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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

THE CLASSIFICATION OF INSECTS ON THE CHARACTERS OF THE LARVA AND PUPA.[1]

CHARLES T. BRUES.

Knowledge of the preparatory stages of insects is still very fragmentary in comparison with the much more extensive information available concerning the comparative structure and classification of the imaginal forms. These developmental stages are of great interest, however, and have been neglected only for practical reasons incident to the vast extent of insects as a group, and the impossibility of correlating the adult and preparatory stages without the laborious process of breeding each individual species.

A comparative study of insect larvæ and pupæ throws much light on the origin and development of metamorphosis, a condition which reaches its climax in the highly specialized insect. It is also essential to an understanding of the varied adaptations of insects, many of which appear in such a form that their biological significance cannot otherwise be recognized. The independent adaptations of insect larvæ are peculiar phenomena, which must aid greatly in the application of the biogenetic law to insects; in fact any attempt to correlate insect ontogeny with phylogeny must be undertaken with careful attention to the characteristics of larval development. Such functional and structural modifications in larval insects, have I think, not received the attention which they deserve. They have usually been considered as phenomena without close counterpart among other animals, or at least as such an exaggerated expression of metamorphic development that they could not readily be reduced to simpler terms. Many entomologists have of course traced the very evident progress of metamorphosis, from the rather gradual series of changes shown in the ontogeny of the more primitive insects, to the highly adaptive, saltatory process exhibited by members of several of the greatly specialized orders, and have interpreted metamorphosis on a physiological basis.

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 157.

Before entering into a discussion of those characteristics of insect larvæ and pupæ which are of interest from a taxonomic standpoint, I hope I may be pardoned for calling attention to several oft-repeated groups of facts relating to insects in general. Insects stand apart from other animals in several particulars. They include a great wealth of species, known mainly in the adult stage through the labors of a large number of systematic entomologists. In spite of the fact that these workers have described an almost unbelievably large number of living species, the insect fauna of every zoölogical region is still so imperfectly known that, not only are many new species and genera discovered each year, but entirely new families and even new orders, are most unexpectedly brought to light. Thus, at least two new orders of insects have been found within the past ten years, while in the same period three very clearly defined families have been added to the well-known order Hymenoptera. The great variety of insects is undoubtedly due to their having appropriated practically every sort of environment. Thus, aside from the large number of terrestrial forms adapted for aërial life in their perfect condition, many are amphibiotic, or even purely aquatic during either a part or during the whole of their life-cycle. Others have become epiphytic or epizoic, with the great modifications attendant upon this mode of life. Some are internal parasites, most generally of other insects, either in their preparatory stages, or more rarely, during their entire life. Highly predatory species, comparable to the birds and beasts of prey, are also numerous, and examples of many other striking methods of life will readily occur to one after a moment's thought. The comparative anatomy of insects may be studied much more readily than that of most other groups of animals, since the skeletal elements are external in position, where they may readily be studied in the most minute details. This condition finds no other close parallel in other groups of animals. It is true that the shells of molluscs, the tests of echinoderms, or the external armor of reptiles present features in common, but they do not exhibit in combination, the high degree of adaptiveness and the great variety of modification seen in the exoskeleton of insects. Crustacea make the closest approach to this condition, but these animals are greatly restricted in many ways by their more uniform aquatic environment.

It is, of course, in relation to their metamorphosis, with the interpolation of such dissimilar preparatory stages, that insects find one of their greatest opportunities for functional and structural modification. In consequence, we find that the larvæ exhibit a greater degree of plasticity than do the imagines. Apart from the comparatively short period during which the adults exercise their reproductive functions, the important activities of the higher insects are restricted to the usually much prolonged period of larval development. This period is largely concerned with the growth through which adult size is reached, and growth practically stops in almost all insects at the completion of the final larval instar. Although the beginnings of the purely imaginal organs may appear often quite early in larval development, they do not assume much physiological importance until the pupal stage, at which time the relations of the insect and its external surroundings suddenly decrease almost to the vanishing point, and all activities are closely bound up in the profound internal changes necessary to bridge the gap between larva and imago.

It is, therefore, clear that the trophic functions dominate the larva as most essential to its growth, and it appears that the characteristics of the larval stages may usually be interpreted with this in view. That the larva shows the greatest plasticity will I think be evident from an examination of the structure of the larvæ of the several groups. The pupa on the other hand is the most uniform stage, a condition which may be readily attributed to its much reduced relations with its environment and the slight peculiarities of some pupæ are usually directly correlated with the few activities which they do exhibit toward external conditions.

The modifications of the imaginal insect naturally show much greater complexity, and are far less easily to be correlated with any group of activities. It can be accepted as a general principle also, that they are in great part seemingly less directly due to the environment, excluding, of course, certain features like those related to oviposition, hypogæic life, etc. In spite of many apparently exuberant structural characters, frequent greatly exaggerated development of certain parts, and occasional extreme secondary simplification, the imaginal condition is far more stable. This is most readily appreciated by taxonomists, who experience far less difficulty in delimiting large groups on the basis of adults than they do in attempting a similar treatment of larvæ.

The wide applicability of recapitulation and the biogenetic law to the ontogeny animals in general is so clear that its importance as a fundamental principle cannot be discounted. The exact nature of the parallelism between phylogeny and ontogeny has always been the basis for divergent views on the part of biologists, who have readily accepted its existence, and its origin as a part of the evolutionary process. It has been applied with much general success to the embryogeny of the vertebrates and of many invertebrates. In many cases of invertebrates that exhibit striking metamorphosis, like crustaceans and echinoderms, at least some features of the post-embryonic development fall readily in line with the more continuous process of recapitulation in the vertebrate embryo.

It is with the metabola among insects that we find the intiation of an almost entirely discontinuous process in development, interpolated between the embryonic development and the sexually mature condition. This is most strikingly illustrated by an insect such as the common housefly. During the embryogeny, the rudiments of antennæ, legs and other appendages appear, and give every indication that they will pass over continuously into the homologous parts of the imago. During these stages the embryo undoubtedly reproduces what we consider to have been the original condition in the ancestors of insects, particularly in reference to the metameric disposition of the parts and appendages of the head. In a more generalized nsect we find the imaginal form evolved directly from these initial stages. In the fly, however, as in other specialized insects we find the newly hatched larva almost destitute of morphological indications of most of the appendages which reappear much later in larval life as histoblasts that represent the rudiments of the imaginal appendages.

However, we may regard the interpretation of the biogenetic law as applied to other animals, it is evident that many insects exhibit a very distorted development when viewed from this standpoint. This matter has been treated already by Janet in his "Ontogenèse de l'insecte" ('09). The taxonomist, who is continually brought into contact with the permutations of various single characters, soon begins to regard species as made up of, or at least most easily definable, by series of single clear-cut characters. His attitude is also closely parallel to that of the geneticist who finds that he must attack his problems through the behavior of unit characters. Cope ('95) early called attention to the dependence of the taxonomist upon single characters in his discussion of the biogenetic law as applied to vertebrates.

In a series of papers, which have been gathered together in his "Biologie Générale" ('11), Giard ('92–'05) described certain peculiar phenomena of development which he termed pœcilogony. Among several widely separated groups of animals, certain isolated species show modifications in their ontogeny whereby two widely different types of development give rise to individuals belonging presumably to the same species. This appears frequently to be independent of any alternation of generations and occurs in forms of many well-known genera. Giard regards some cases of pœcilogony from the standpoint of their probable origin as seasonal pœcilogony, geographic pœcilogony or as ethological pœcilogony. How far these phenomena can be regarded as the expression of a single principle is somewhat doubtful, since the significance of the alternation of generations that occurs to such a widely different extent in several groups of insects is by no means clear. Also, de Meijere ('10) and others have shown that the polymorphism of certain Lepidoptera follows Mendel's law in inheritance and that the characteristics of the different forms of female in *Papilio* behave as unit characters.

Such cannot be true of geographic pœcilogony, but however the origin and preservation of the several types of pœcilogenetic development may be explained, the outstanding fact remains that intraspecific pœcilogony occurs. This is of interest in the present connection as it shows that enormous changes in development may readily appear in the early stages of an animal without impressing themselves upon the later stages.

When applied to groups of insects rather than to single species, Giard's views assume great interest from the standpoint of the larval and pupal stages. If two groups of individuals of a single species can reach the adult stage by separate paths, it is seen that we have a much simpler case of apparently similar nature where groups of species have developed highly adaptive methods of development which are far from what might be expected if their development had proceeded along the more conventional lines of ancestral recapitulation.

I shall not digress to attempt any discussion of the origin of metamorphosis in insects nor of the way in which its increasing complexity can be traced in living insects, from those showing its primitive absence to others like the fly referred to above, in which the larva is wholly unlike the imago not only in structure, but in physiology and in its different ethological relationship to a dissimilar environment. From the present standpoint, the hypermetamorphosis of many insects is of especial interest and I shall refer to this subject later.

The properly trained zoölogist is greatly outraged if he should see a paper dealing with the larvæ of certain insects "classified as independent organisms," and will probably go no further to inquire into the viewpoint of the writer. This is very natural when we regard the whole animal and plant world as so closely interdependent that a change in the abundance or distribution of one species soon shows its effect upon a great number of others, whose relations to the first species may not have been thought of previously. Viewed in the light of pœcilogeny, or even after one has worked for some time upon the classification of the highly modified larvæ of some group of insects, it becomes far from an absurdity and the more closely we inquire into the matter the more clearly does it appear that many problems relating to insect larvæ may be profitably dealt with without taking into account the adult insects. This is particularly true with taxonomic studies, for these, when used with due caution, are very valuable in modifying any conclusions based upon studies of the adults.

Even the eggs of insects furnish characters which are suitable for taxonomic treatment. Like the later stages, the eggs commonly show distinctive features in the form and structure of the chitinous shell, aside from the contained embryo. Like the similarly large eggs of birds, they are frequently characteristically colored, although a spotting or color pattern is rather rare among insect eggs. The relation of color development to environment is nicely illustrated by the eggs, as only such as are deposited in places exposed to the light show a color other than that imparted by the whitish or buff-colored yolk. The color often changes where the shell is transparent, as the developing color of the larva is to be seen through the shell. Whether the color of insect eggs may be interpreted on the basis of protective or warning color seems very doubtful. In certain bright yellow or orange eggs, as for example those of many chrysomelid beetles the color may be due to shell color or only to the brightly colored yolk. There seems not to be sufficient uniformity in egg-color, however, to attempt any explanation of its probable function.

The eggs of Lepidoptera, especially those of the butterflies, have received more attention than those of any other insects. Externally these are commonly of unusually complex sculpture, with surface reticulation or areolation, sometimes assuming the form of longitudinal and transverse ribs. Scudder ('89) has shown the possibility of classifying the eggs of some of our North American butterflies, and Tutt ('05–'06) gives an interesting résumé of the egg-structure of some of the groups of British butterflies. According to Tutt, the eggs of the various species of any given family are almost always similar, although varying in detail in the several forms. Thus, the Thymelicid eggs are flat with three axes of different lengths, differing from those of the other groups which are circular in horizontal section. In the Urbicolid skippers they are somewhat more than hemispherical; in the Lycænids shallow, flattened, tiarate or echinoid;

in the Papilionids and some Satyrids almost globular; in the Pierids, long, slender and spindle-shaped; in the Nymphalids, somewhat barrel-shaped with projecting ribs reaching from base to apex, etc. In some butterfly eggs there is a well marked lid, defined by a weakened line in the shell, along which the shell ruptures on hatching, causing the lid to separate from the remainder of the shell. In the Hemipterous family Pentatomidæ a similar lid occurs, along the margin of which is a circle of spines or thorns. When the eggs of insects are better known, they will undoubtedly prove of great interest from a taxonomic standpoint. Incidentally, it is of more than passing interest to note that these organisms in such an early stage of ontogeny show visible external characters by which not only larger groups may be delimited, but which serve to distinguish the numerous species of some groups. In this connection, it must not be forgotten, however, that the egg-shell is really formed by the mother and is hence a direct product of the adult insect and not of the embryo, so that its high specialization cannot be attributed to any differentiation of the embryo. In this sense we are dealing with a matter of entirely different significance from that now to be presented in relation to insect larvæ.

Insect larvæ may be separated into several rather clearly defined types, and in at least one of the extensive orders, the larvæ of the great majority of the species included may be referred to a single, quite consistent type. Without question the most primitive of these is the campodeiform larvæ so named in recognition of its similarity to the imago of *Campodea*, a genus of Apterygote insects. Campodeiform larvæ appear with many modifications in several widely separated orders of insects. A second, quite consistent type, is caterpillar-like or eruciform, and larvæ of this kind prevail almost universally in the Panorpatæ and several orders which appear to have been derived from a Panorpate ancestral type. Various other types are also easily to be recognized, most of them of quite restricted occurrence and characteristic habitus. A vast residuum are either highly specialized in structure or greatly simplified through the loss of appendages and the acquisition of a vermiform body. These latter, so-called apodous larvæ, although quite uniform in gross external appearance represent numerous independent lines of development.

In almost any group of insects there is easily seen the great ease with which adaptations in form appear, most commonly in response to the requirements of alimentation and respiration. Among these might be mentioned many grotesque modifications of the trophi of entomophagous parasites, the posterior closure of the alimentary tract, the great flattening of the body in many subcorticolous species, and the development of respiratory tubes, gills, etc., in aquatic forms. In details, the active, free-living types of larvæ naturally furnish the most striking and clear-cut characters, while those of sessile, or at least inactive habits show such a reduction or complexity in structrue, that they tend to converge toward a common type. There are notable exceptions to this last statement, however, as for example among the maggots of the higher Diptera to which I shall return later.

During recent years the larvæ of mosquitoes and those of some other Nematocerous Diptera, have been examined with perhaps greater care and thoroughness than those of any other insects. Were it not for this, no one should think of considering the highly specialized larvæ of this group before dealing with those of the more generalized insects. Mosquitoes are well-known as remarkably adaptive insects in many respects. The adult mosquito (at least the female) is phlebotomic and depends upon the higher vertebrates for its food. It further shows a complex relationship to its vertebrate food-reservoirs through the medium of certain Protozoan parasites. The malarial parasites of man undergo their definitive life cycle in the mosquito and the same is true of various other similar Protozoa parasitic in the hosts of other mosquitoes. As is well known to every one, the larvæ of mosquitoes are very active aquatic creatures of very peculiar, although quite uniform conformation. It is perhaps not so well known that many of the species are restricted quite closely to certain places, *e. g.*, ponds, pools, rain-barrels, brackish water, permanent water, temporary water, etc., in such a way as to show that they thrive ordinarily only under quite specific conditions. Nearly all are surface breathers and are equipped with a breathing tube into which the main lateral tracheal trunks extend, and which they thrust through the surface of the water for respiration. The mosquito larvæ is by no means unique among the lower Diptera in the general plan of its body although it is the most highly specialized in several respects.

A close morphological study of the larvæ of a large number of mosquitoes has brought to light many interesting facts. Of approximately 400 species known from North and Central America, the larvæ of a very large majority are known and have been carefully examined. Comparative descriptions of a considerable number of these were published in 1906 by Dyar and Knab, and in their monograph on mosquitoes ('12–'17) Howard, Dyar and Knab have included a complete account of all of the known larvæ from this region. In the earlier paper ('06) the statement is made that "we are compelled to the conclusion that specific limits are more sharply defined, or at least more readily appreciable, in the larvæ of Culicidæ, than in the adults, although generic limitations are less sharply drawn." There seems to be no reason to change fundamentally this statement although it appears from the later and more extensive publication that its authors have been unable to find satisfactory diagnostic characters on the basis of larval structure for a small percentage of the recognized species. Such characters as they do employ in their keys are readily perceived and the identification of larval mosquitoes is far easier than that of the adults, at least for those not thoroughly familiar with the group.

The two (or sometimes, three) subfamilies of the Culicidæ as at present constituted, are readily separable on the basis of larval structure and the same is true of the two tribes of Culicinæ (true mosquitoes). So far as one may divest one's mind of preconceived notions, it would appear that the genera of the subfamilies would have been segregated in much the same manner if the adults had remained unknown. It appears quite certain, however, that the division of the tribes would have been different to some extent, as the Anopheline mosquitoes present a striking difference from the other members of the tribe in which they are placed, on account of their extremely short breathing tube. It must also be admitted that in the adult condition they are a very clearly characterized group.

From the keys included in the monograph, it appears that in a number of cases, the larvæ do not fall readily into the linear arrangement of species adopted.

From the foregoing much abbreviated and necessarily incomplete account, it is very evident that the larvæ of mosquitoes exhibit morphological differences approximating in extent and fixity those characterizing the adults of the several species, and that a scheme of classification based on larval structures runs quite parallel with one based upon the imagines. In the case of these insects the larvæ show little differentiation in color and other superficial details, and the recognition characters are essentially those of structure.

In the pupæ of mosquitoes the development of specific, generic or tribal character is very limited and the identification of species in this stage is not satisfactory. This is quite like the condition in other groups of insects, although mosquitoes and related forms are almost the only insects with an active pupa. The activity of the pupa even in this case, is of course only a limited retention of the great activity of the larva, necessitated by its adjustment to aquatic life. The mosquito pupa breathes in a manner similar to the larva, by a pair of spiracular tubes that are thrust above the surface of the water, and although these organs emanate from the anterior part of the thorax in the pupa, this stage must remain highly active in order to perform its respiratory function.

Much attention has also been given to the systematic description and classification of the larvæ and pupæ of the other more generalized families of Diptera related to the mosquitoes by Osten Sacken ('62), Brauer ('83), Hart ('95), Johannsen ('03–'05) and numerous other entomologists. Quite recently Malloch ('17) has gathered together this information relating to American species and supplemented it with much new material. In these other families, the larvæ of the numerous species may very generally be easily distinguished and tabulated upon much the same type of characters as have been used for the mosquitoes. The genera and families are also separable on the same basis although the tabulation of the families is more difficult, indicating that the natural affinities are well indicated in the preparatory stages. In fact the correlation between the two is very close, even to the difficulty of distinguishing two closely related groups. This is illustrated by the families Chrironomidæ and Ceratopogonidæ, separated in the imagines only by the blood-sucking habits and consequently modified mouthparts of the last family, and not readily characterized in the larval stage by mutually exclusive characters. The same condition is true of two divisions (Tipulidæ and Limnobiidæ) of the old family Tipulidæ. In all of these families more difficulty is met with in classifying the pupæ, as we have seen to be the case in the more completely known family of mosquitoes.

All of the Diptera so far referred to are not so highly specialized as many other families of the order, although among insects in general the Diptera must be regarded as structurally the most specialized group. Those which we have described, known as the Nematocera, retain in the larval condition some features of the generalized insect, notably in the well developed, free head, with the mandibles moving in opposition in a horizontal plane. To disregard for the moment the families of somewhat intermediate structure, and to turn to the higher Diptera, or Cyclorrhapha, we find larvæ of a very different type. They are maggot-like, microcephalic, with the head not differentiated and without appendages, with a simple internal framework, the cephalophyrangeal skeleton, that bears vertical, hook-like, non-opposable mandibles. Through almost the entire series of families, and in innumerable species, the larvæ present almost exactly the same appearance, with only the occasional development of some striking features (*e. g.*, in the genus *Fannia*, which has some elaborate tegumentary appendages). In these larvæ, only the posterior pair of spiracles are functional. These, however, are large, and each is provided with three slits. The form, size, and position of the spiracles, and the form, size, position, length, curvature and microscopic structure of the slits present such a complex series of permutations, that these organs in connection with certain cuticular structures, usually associated with the spiracular plates, serve for the identification of very many species. The value of these structures has long been recognized as valuable in classification and they have been carefully figured and described for a large number of species. Certain authors, notably

Portschinski (several papers), Osten Sacken ('87), Blanchard ('93–'96), Banks ('12), MacGregor ('14), and Rodhain and Bequaert ('16) have dealt with some of these larvæ in a way which is of interest in the present connection. As the specific characters in these flies relate to so few structures, it is very difficult to delimit the larger groups such as families, although the species appear in great measure to have individual characteristics of easy recognition.

The classification of the several families of flies related to the Muscidæ has been the cause of much controversy among entomologists and there is no question concerning the advisability of utilizing the larvæ in any attempt to elucidate their natural affinities. Not excepting any animals, the species of some of these families are distinguished by the most minute characters, so slightly do the adults vary in structure. In very many cases, it is already known that the larvæ show more striking differences, but it would be unwise at the present time to do more than to predict that a comparison of adult and larval structure and of habits and behavior in these insects, which appear to be undergoing a rapid evolution, will lead to results of great interest.

Another group of insects in which it is possible to trace the probable course of larval modification in the form of several series is seen in the Trichoptera, Lepidoptera and Hymenoptera. These orders appear to have been developed rather directly from a group similar to the Panorpatæ or Mecoptera which is represented, although not very extensively in the living fauna. In these several orders the larva is primarily of the eruciform type, but becomes apodous in some cases, more generally so in the Hymenoptera, where only a small series of families retain the caterpillar-like larva.

Of all larval insects, the caterpillars of various butterflies and moths are best known to the casual observer and they have long attracted the attention of naturalists. In general form they are remarkably uniform, with long cylindrical body, well developed head capsule and trophi, functional thoracic legs and a series of prolegs on certain segments of the abdomen differing greatly in structure from the thoracic ones, but homologous with them. In only a few Lepidoptera do the larvæ become apodous or nearly so. The majority of caterpillars occur upon their food plants in exposed situations, and as they are very helpless creatures, have developed in many cases elaborate spines, tufts of bristles, or poisonous hairs which are undoubtedly protective adaptations. Many of them are also remarkably colored, presenting shades and patterns that may be readily interpreted on the basis of the theories of protective resemblance, warning color and mimicry. Consequently, at first blush these larvæ would not appear to show any consistent characters applicable to their classification on a rational basis. In 1886 W. Müller found that the position of certain primary body-setæ might be utilized for the purpose of classification. Dyar ('94), Forbes ('10), Tsou ('14), and most recently Fracker ('15), have elaborated and modified this idea and it is now applicable in a most satisfactory way throughout almost the entire order. Thus we find that the arrangement of these setæ follows a certain plan, that the individual setæ are homologous, and that the modifications present in specialized families may be derived from those of more primitive groups. Still more interesting is the fact that very young larvæ of all groups tend to approach more closely to the primitive arrangement than the larvæ of later instars. We find thus quite evidently that in ontogeny the caterpillar reproduces certain changes in setal arrangement that have occurred in the phylogeny of the group. Fracker has called attention to this as he found it to occur quite consistently, although he fails to point out some of the interesting conclusions to which it may lead. This is really a beautiful case of the morphological independence of the larval state in insects. These characters, which are of a purely transitory nature, have never been in any sense adult structures, yet they have so thoroughly impressed themselves on the species, that they repeat their history in a very specific way in the development of the individual caterpillar.

On account of the small size and relatively unimportant function of the setæ, their use in classification is greatly enhanced. Thus the larger groups of Lepidoptera can be delimited more easily from a study of the larvæ than can those of most other insects, so far as our present knowledge extends.

Until very recently the pupæ of the Lepidoptera have not received any general taxonomic treatment, although those of certain groups had previously been given attention by Packard and others, and those of many species had been described. As we should expect from the quiescent condition of the pupa it presents few characters other than those derived directly from the more or less fully developed external structure of the contained imago. Indeed, the cremaster, certain locomotory spines on the abdomen and tubercles on the thorax, are practically the only characters present which may be regarded as independent pupal modifications. Consequently, they are of much less interest than the larvæ, although Miss Mosher ('16) has been able to base, on the characters of the pupæ workable keys for identification of the families and of various groups of genera.

The Hymenoptera are perhaps the most remarkable group of insects in relation to their post-embryonic development as they are also in several other respects. In this group, as already stated, the larvæ of only the most generalized families, the saw-flies, retain the typical eruciform larva. They are in general appearance very much like Lepidopterous caterpillars, although differing at once in the minute structure of the legs and in the number of the abdominal ones. I am not aware that anyone has attempted a general classification of the saw-flies on the basis of larval structures although the larvæ of a large number of species have been described, and a very interesting account of the external anatomy has been given by MacGillivray ('13). Many are leaf-feeding in habits and show color patterns similar to those of the true caterpillars.

The larvæ of all the other groups of Hymenoptera are very greatly modified and hypermetamorphosis occurs in a number of cases.

Comparatively little is known of the larvæ of the several families of parasitic Hymenoptera, but those of some of the members of this group are very remarkable. In the later stages, nearly all are apodous, of very simple form, and without noticeable peculiarities. In the earlier stages, however, many strangely modified types are known which appear to be highly adapted to their mode of life. Some of these in which marked hypermetamorphosis occurs may be illustrated by Synopeas which has been described and beautifully figured by Marchal ('06). Here the first larval stage which he has termed the Cyclops-larva is composed of an enormous cephalo-thorax, bearing a pair of absurdly enlarged jaws, and a slender abdominal portion. In the course of later development the abdomen enlarges, the jaws are cast off and the later stages assume the obese form characteristic of many larvæ of the higher Hymenoptera. Somewhat similar cyclopoid or highly modified larvæ are known to occur in a number of widely scattered genera and it will be doubtless found that this stage will greatly supplement any knowledge concerning the natural affinities of the very numerous and closely similar species. It is probable also that the structure of the larvæ will be found to reflect much more clearly the diversified behavior of these insects than does the structure of the imago.

As a rule the larger species of the parasitic Hymenoptera show less modification, but in a few of the small number of forms that have been studied, the early larvæ is provided with unusual structures of one kind or another. Most frequently the mandibles are noticeably enlarged.

Some of the members of this same group exhibit a very interesting phenomenon in the development of an early larva of typical campodeiform appearance which simulates very closely the first-stage larva of certain Meloid beetles and of the Strepsiptera. This hymenopterous planidium is, of course, not by any means identical with the triungulin in the other two orders, but presents many features in common with it. It will be noted also that it is the early larva and not the later one that is of the campodeiform type. The reason for the presence of a very active young larva is clear in all three cases, as the larva actually finds its own host, and is dependent upon its own resources at this early age. Here it is very evident that the planidium and triungulin have been interpolated in the larval development just as the whole larval stage has been added to the life-cycle of the metabolous insects. Their presence, especially in Hymenoptera is quite secondary.

Aside from those of the saw-flies, the larvæ of ants should be more susceptible to taxonomic treatment than those of other Hymenoptera. In general, these do not possess such remarkable modifications as occur in some of the parasitic families, but the characters which they do have are apparently of such nature as to be suitable as a basis for classification.

The larvæ of the Coleoptera are probably, on the whole, more generalized than those of any other order of holometabolous insects. At any rate, the larvæ of the most primitive families, which are included in the suborder Adephaga, are campodeiform and in many cases similar to the adults. As in all larvæ, there are, of course, no wings and the trophi, antennæ, eyes and legs are much reduced and simplified. In the suborder Rhyncophora or snout-bettles, the larvæ are legless and much simplified in structure, and this condition prevails in a couple of other, evidently related families. For a long time the larvæ of beetles have attracted the attention of entomologists on account of their greatly diversified structure and interesting adaptations. At about the same time, two European zoölogists, Perris ('76) and Schiødte ('76–'83) published elaborate and beautifully illustrated systematic accounts of beetle larvæ, and made available a vast amount of material relating to these insects. Comparatively little was added to this for many years, but interest is again awakening in the matter.

The work of Schiødte and Perris shows very clearly that a complete classification of many families of beetles on the basis of the larvæ will be possible. As is well known to all entomologists, the larvæ of a good many groups are recognizable at a glance, from their general habitus alone. Of these one series representing the lady-birds or Coccinellidæ has recently been treated by Böving ('17) in a systematic way. These larvæ are active, predatory forms with well developed legs and with various tubercles, spines or processes developed on the thorax and abdomen. Böving has been able to develop a system of classification based upon many characters which appears to be very satisfactory, although limited as are all studies of this sort by the great difficulty in obtaining extensive series of larvæ that are accurately correlated with the imagines.

A very different type of Coleopterous larvæ occurs in the family Cerambycidæ, or longicorn beetles. In this group, the larvæ are legless, cylindrical creatures, undergoing their entire development in the woody tissue of various shrubs and trees.

Like all larvæ of similar habits they are of uniform white or buff color, except for the pigmentation of certain parts and appendages of the head. One could scarcely find any series of insect larvæ of more uniform appearance, yet we find that the structural and color characteristics of the adults of this enormous family are remarkably diverse and striking. In connection with their general work on beetle larvæ, Perris and Schiodte have examined some representative longicorn larvæ, but quite recently, two American entomologists, Webb ('12) and Craighead ('15) have attempted to classify these larvæ on structural characters. Although they have been restricted to a small number of details, mainly confined to the head, it appears that most of the genera may be distinguished more or less satisfactorily. The subfamilies (or families as they are called by Webb) appear also to be easily recognizable in the larvæ. If this proves still to be true as the larvæ of more genera and species are made known, it will be of particular interest, since such divisions are difficult on the basis of the imagines. We have seen that in most groups of insects the larvæ are generally less useful for the delimitation of larger groups than the adults.

Mention has already been made of hypermetamorphosis, and its occurrence in certain beetles noted. A closer examination of the larval stages of the Meloidæ shows, however, such a series of very evidently adaptive forms, that I cannot refrain from considering these somewhat in detail. The preparatory stages of various Meloids live upon the egg masses of certain locusts and within the larval cells of solitary bees, and several very distinct larval types succeed one another in the course of development. The parent beetle does not deposit her eggs directly in the materials which the larvæ is destined to devour, and the newly hatched Meloid larva is a tiny, active, long-lived creature that goes in search of its proper environment either by seeking out the locust eggs, or by attaching itself to the bee and thus being carried to its food. The triungulin is thus well-fitted to fulfill its mission and so far as its structure is concerned, presents nothing unexpected, for it is of the typical campodeiform type, widely distributed among insects. In the case of *Epicauta*, parasitic on locust eggs, shortly after reaching its food-supply, the larva molts and assumes a second form with shorter legs and mandibles and soft skin; it is still distinctly of the campodeiform type, however, and similar to the larvæ of a number of other rather primitive families of beetles, and hence termed "caraboid" from the family Carabidæ. After another molt another larva appears, very similar to that of the family Scarabæidæ, and suitably known as the scarabæoid larva. The next stage is formed in a smooth cavity in the soil and is characterized by a more or less rigid, curved body; it is known as the coarctate larva and usually undergoes hibernation. In the spring, the next larva is a return to the Scarabæoid type which does not feed, but burrows about for a time and finally pupates after the manner of other beetles.

It is very evident that the several larval forms are beautifully adapted to the varied conditions which the larva has to meet during its development. They also follow a general course of degredation and loss of activity as their life cycle, reproducing in a general way the evident steps taken by evolution in the transition from the campodeiform to the apodous type. There is one anachronism, however, in relation to the scarabæoid larvæ. If, as appears to be true, this is really similar to the larva so named in the family Scarabæidæ, it can not bear any genetic relationship, since the Lamellicornia, to which the Scarabæidæ belong is a very highly specialized group which could not be derived from the otherwise greatly specialized Meloidæ that are the offshoot of another group of beetles. Likewise we cannot imagine a derivation in the opposite direction. We are forced, therefore, to the conclusion that this specific larvæ is only a response to similar stimuli in the environment in the two insects. Such a case serves well to illustrate that in larvæ as in adults the appearance of similar structures through convergence may easily lead to misconceptions. This example is more striking than the independent appearance of triungulin larvæ in widely separated groups, for as we have seen the triungulin is a very generalized larva of the campodeiform type.

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THE INFLUENCE OF GLANDULAR EXTRACTS UPON THE CONTRACTILE VACUOLES OF PARAMECIUM CAUDATUM.

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Contractile vacuoles have long been puzzling morphological elements in the Infusoria. In general they may be said to be cavities in the protoplasm destined to expel by contraction the liquid which they accumulate. They are present in all ciliated infusorians except a few salt water and parasitic forms, but since this paper deals primarily with the physiology of the vacuoles in *Paramecium caudatum* a description of their structure will be limited to this species.

Normally *Paramecium caudatum* possesses two contractile vacuoles situated in the ectoplasm, one at the posterior end and the other about mid-way between the anterior and posterior ends. Cases have been noted by Hance (11) where a race seemed to show a tendency toward multiple vacuoles, and by Shumway (26) where the individual appeared to be stimulated to the production of extra vacuoles by some abnormal physiological influence. Invariably the vacuoles are arranged in a straight line. Each vacuole is composed of a central reservoir with peripheral canals radiating from it, the number of canals varying with the individual vacuole. The mechanism of contraction is as follows. The canals swell slowly, then discharge their fluid contents into the reservoir, which in turn contracts, expelling its contents to the outside through an excretory pore. In systole the reservoir disappears completely from view, leaving clearly visible the rosette of canals slowly filling again. A new cavity must be formed by contraction of the canals in the protoplasm which has replaced the old reservoir. The outline of this new reservoir is irregular at first, but gradually assumes the shape of the circular vacuole. The canals are simple afferent tubules which pulsate twice to every contraction of the vacuole and remain constant in form and structure. While the reservoir appears always in the same place, its walls are formed of new substance at each pulsation. There is much controversy as to the exact nature of the enveloping material, Kent (16) cited Carter, Ehrenberg, Siebold, Claparede and Lachmann as believing in the presence of a morphologically distinct investing membrane, while Maupas (20), Ehrmann (8), Degen (6), and Minchin (21) upheld the conflicting view that the vacuole is simply a drop of watery fluid lodged in, and bounded by a more viscid protoplasm. Maupas found the wall comparable to that of a soap-bubble, since according to him, the viscid protoplasm is distended by increased pressure until it breaks at the weakest point, the excretory pore. The rhythmic contractions he believed due merely to the innate irritability of the protoplasm in response to a specific stimulation. According to Bütschli's alveolar theory of protoplasm (3) the behavior of the vacuoles is explicable entirely by the physical laws of fluid masses. Andrews (1) developed the application of this theory, describing the rhythmic variation of viscosity in the pellicular membrane of the contractile vacuole:

"At times in the rhythm the vacuole actually disappears, and during collapse the pellicular substance becomes so completely relaxed, so fluid, that it mingles with the interalveolar substance of the surrounding protoplasm. As the membrane reforms after such collapse it thickens and is gradually augmented by interalveolar stuff. As a contractile pellicle it is subject at all times to the same flow of its substance as is the surrounding network, and is at times so modified in structure and reaction as to form an area of organized physiological reaction."

Claparede and Lachmann believed that the vacuole has no intercommunication with the surrounding medium, while Carter and Lankester (18) held that the vacuole discharges externally at the time of collapse. Wrzesniowski (30) Maupas (20), Butschli (3) and Hance (11) definitely described the pore-like character of the communication. Jennings (14) proved this inter-communication conclusively by demonstrating the discharge of the vacuole into a surrounding medium of dilute india ink.

The theory of Haeckel (10) that the pulsating vacuoles are derived from the simple food vacuoles was questioned by Maupas (20), who declared that the two vacuoles have but one characteristic in common,—the absence of a true limiting membrane. In all other characteristics they differ completely. The fluid content of the contractile vacuole contains the soluble waste products of metabolism, while that of the food vacuole is a plasma or cellulose sugar in which digestion can take place. According to Maupas (20) the contractile vacuole is a special physiological adaptation of the protoplasm and does not depend phylogenetically upon any other structure.

Opinions are even more contradictory in regard to the special function of the vacuole. Hartog (12) and later Stempell (28) emphasized the importance of the vacuole as an adjustor of osmotic equilibrium. It is a physical necessity to the naked cell living in water since it prevents overdilution and ultimate destruction of the protoplasm. Others ascribed to the vacuole the additional functions of circulation, respiration, and excretion. No one can doubt its assistance in general circulation. Kent (16) mentioned that Lieberkuhn, Claperede and Lachmann even considered it a rudimentary heart. In respiration it serves according to Haeckel (10), Maupas (20), Butschli (3), Ehrmann (8) and Stempell (28) merely as an agent to remove the end products of oxidation. The fluid which accumulates in the vacuole has previously circulated through the organism, and must therefore have lost the feeble quantity of oxygen which it held in solution on entering the protoplasm through the gullet or the periphery. The vacuole may be said to receive all the soluble end products of general metabolism. Schmidt (16) found the vacuole comparable in function to the renal organs of higher animals, especially the excretory water canals of *Turbellaria*.

Rossbach (24) first showed conclusively that the rhythm and size of the vacuole vary with the physical and chemical properties of the surrounding medium. Since then a considerable amount of work has been done on the physiology of these primitive organs. The pulse frequency, or the number of seconds elapsing between two successive pulsations presents under normal conditions a constant average among individuals of the same race.

Ehrmann (8) set this normal average as twelve for *Paramecium.* Hance (11) found that with multiple vacuoles there is an increase in pulse frequency toward the posterior end. The observations reported in this paper will show however that this may occur also in normal individuals. Moreover, the reverse is frequently true, that the anterior vacuole or vacuoles may pulsate more rapidly than the posterior. Rossbach (24) experimenting with temperature changes, gases, chemical solutions and electricity found that rate of contraction increased with rise in temperature and with most chemical reagents. Dilation with retardation was produced by neutral substances and alkaloids, and no effect whatever was noticeable with currents of oxygen or of electricity. Korentschewsky (17) confirmed this retarding effect of alkaloids.

Degen (6) published an exhaustive treatise on the physiology of the vacuole. Continuing the temperature experiments of Rossbach, he established a maximum and minimum for the organism. Around zero degrees he found a great variation in pulse frequency, but a generally slow contraction with dilation of the vacuole. At about three, Centigrade, dilation ceased and the pulse frequency became more and more rapid until the animal plasmolysed at about thirty-four degrees. Doflein (7) gave the optimum temperature as thirty to thirty-five degrees for *Paramecium.* In contrast to Rossbach's observations (24) Degen found that a current of oxygen produced a pulse quickening to compensate for the increased output of carbon dioxide produced by the accelerated metabolism. Hydrogen, however, retarded the pulse, dilated the vacuole and caused dissolution in four or five hours. When subjected to carbonic acid gas the vacuole dilated with an increase in pulse frequency and dissolution occurred in two hours. Minchin (21) observed that carbon dioxide diminished the frequency. This seeming contradiction may be due to the specific resistance of the organism in media saturated with the gas. Jacobs (13) believes that the organisms in such environment develop a remarkable resistance to other chemical factors. That pulse frequency often may be attributable to the concentration of the surrounding medium was shown by Degen in the effects of chemical solutions of varying strength.

An important factor is the specific permeability of the osmotic membrane. Degen found that alcohol, ether, acetone and chloroform had a rapid lethal effect but little direct influence on the vacuole. He concluded from his various experiments that the vacuolar membrane has a remarkable physiological, but no morphological differentiation from the surrounding protoplasm. The frequency of the pulse he believed conditioned by the water intake and its osmotic value relative to the environmental fluid.

It would seem that any agent as powerful as the glandular extracts should exert a strong influence upon the rhythmic contractions and perhaps by means of a specific influence give some important information as to the function of this primitive organ. Some work has been done already on the effect of the extracts upon the general metabolism of protozoa. Nowikoff (22) found that thyroid extract increased the division rate in *Paramecium*. Extract of hypophysis had no such effect. Calkins (4) obtained no reactions with pancreatic extract. Shumway (26) confirmed Nowikoff's observations, finding that thyroid extract was the only glandular product producing any significant increase in division rate. He also noted a tendency toward the formation of extra vacuoles and an increase in pulse frequency. Chambers (5) found that a solution of suprarenal extract with a basic fluid of either hay infusion or malted milk produced an increase in division rate, while a solution of pituitary, Armour & Co. tablets, produced no influence with malted milk and only a very slight increase in division rate with hay infusion. A mixture of pituitary and suprarenal solutions in the basic food substance appeared to reduce the division average.

In October, 1918, the following experiments were started to determine the specific influence of adrenalin, pituitary substance and pineal gland extract upon the contractile vacuoles of *Paramecium caudatum*. Individuals for the experiment were taken from wild cultures just introduced into the laboratory. First a number of observations were made upon pure line cultures to determine whether or not there might be, (1) any significant variation in the pulse frequency of individuals in different pure lines kept in the same physiological environment, (2) any significant similarity in the pulse frequency of individuals in the same pure line kept in the same physiological environment, and (3) any significant diversity in the pulse rate of individuals from a wild culture kept under approximately the same conditions in the laboratory. Two pure lines—A and B—were started on a depression slide, each being given 2 drops of distilled water and one drop of 1 per cent. malted milk solution daily. On another depression slide were placed drops containing individuals taken from a wild culture. These were given the same diet. Both slides were kept in a moist chamber at room temperature. On the third and fourth days averages were made of the pulse rate of four individuals from each group. It was found by repeated experimentation that when the animals were fed at ten o'clock in the morning they usually quieted down at about four o'clock in the afternoon. Various futile attempts were made to quiet them artificially without disturbing the pulse rate, but any methods employed which increased the density or the pressure of the surrounding medium definitely decreased the pulse frequency. The individuals were kept on depression slides and observed under both high and low power. By using a stop-watch an accurate record of the pulsations could be kept. In all cases the pulse rate of both vacuoles was taken, going alternately from one to the other, and recording ten pulsations of each. It would be unwise to observe ten contractions of one vacuole before noting the other because of the rapid effect of any change in physiological conditions. Tables I. and II. give the results of the first series of experiments.

From these records it is seen that the variability in pulse frequency in different pure lines in not significantly greater than the variability among individuals in a single pure line. Taking the anterior vacuole, the average for pure line A is 3.9 seconds more rapid than for pure line B, while the greatest variation for any group within pure line A is 1.6 and for any group within pure line B is 4.3. The same is true for the posterior vacuole. The average for pure line A is 3.5 seconds more rapid than for pure line B, while the greatest variation for any group within pure line A is 1.8 and for any group within pure line B is 4.1. For the wild culture the average of each vacuole presents approximately the mean average of the corresponding vacuoles of the two pure lines, and the variations among individuals from the culture are not so great as the variations among individuals within either pure line. From this we may conclude that there is (1) no significant variation in the pulse frequency of individuals in different pure lines kept in the same physiological environment, (2) no significant similarity in the pulse rate of individuals in the same pure line kept in the same physiological environment, (3) no significant diversity in the pulse rate of individuals from a wild culture kept under approximately the same conditions in

Table I.

Pulse Frequency under Normal Conditions.
(*Given in Seconds.*)

Pure Line A.

1.		2.		3.		4.	
Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.
13.0	11.0	13.0	10.6	9.0	8.0	12.0	11.2
13.0	14.0	13.0	10.6	9.8	8.2	11.4	11.2
13.0	12.5	11.5	13.0	9.8	9.4	12.4	10.6
12.0	11.0	11.2	10.6	10.0	9.2	12.0	10.2
13.0	13.0	11.2	11.0	10.0	9.0	11.4	11.2
12.0	11.0	12.0	10.6	9.6	8.4	11.0	11.0
13.0	12.0	12.0	10.4	9.8	9.2	12.6	11.0
11.5	12.0	11.2	12.0	9.4	8.8	11.0	10.8
11.5	12.5	12.0	12.0	9.6	9.8	10.0	10.4
11.0	10.5	12.0	11.0	9.8	9.0	10.0	12.0
123	119.5	119.1	111.8	96.8	89	113.8	109.6
12.3	11.9	11.9	11.1	9.6	8.9	11.3	10.9

Pure Line B.

1.		2.		3.		4.	
Ant.	Post	Ant.	Post.	Ant.	Post.	Ant.	Post.
17.0	17.0	15.0	17.0	16.6	14.0	10.6	10.2
19.4	18.6	17.0	17.6	16.4	14.0	10.6	10.4
17.0	16.6	17.4	15.0	16.0	14.2	10.4	9.8
18.0	16.8	16.2	15.8	15.0	12.4	11.2	10.4
17.0	17.2	17.6	14.0	17.0	12.4	10.6	9.8
17.6	16.6	16.2	14.0	16.4	15.2	11.0	9.8
17.4	17.6	15.4	14.4	17.0	14.2	10.8	10.2
17.0	17.0	16.0	14.0	16.0	16.6	10.8	10.8
17.0	15.8	14.8	15.6	15.0	15.2	11.2	10.4
17.0	17.0	16.6	17.0	16.0	15.0	10.8	10.0
174.4	170.2	162.2	154.4	161.4	143.2	108.0	101.8
17.4	17.0	16.2	15.4	16.1	14.3	10.8	10.1

Wild Culture.

1.		2.		3.		4.	
Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.
11.4	10.6	14.4	12.6	10.8	11.0	12.8	11.0
12.0	11.2	14.4	12.2	10.4	11.0	12.6	11.0
13.0	12.0	15.0	12.4	11.0	11.2	13.0	11.4
12.0	11.2	14.4	12.4	11.2	10.8	13.2	11.4
12.0	11.6	14.6	13.0	10.8	11.2	13.2	11.4
13.0	11.8	15.0	12.6	11.2	11.8	13.0	11.0
13.0	11.2	15.0	12.6	11.0	12.0	13.0	11.0
13.4	12.0	14.4	12.2	10.0	11.2	12.6	11.2
12.6	12.4	14.4	13.0	11.2	10.8	13.0	11.0
12.0	12.0	14.4	12.2	11.0	11.0	12.8	12.8
123.4	116.0	146.0	125.2	108.6	121.0	129.2	113.2
12.3	11.6	14.6	12.5	10.8	11.2	12.9	11.3

TABLE II.

AVERAGE RATE OF PULSATION FOR EACH GROUP.

Anterior Vacuole.		Posterior Vacuole.	
Pure line A	11.2	Pure line A	10.7
Pure line B	15.1	Pure line B	14.2
Wild Culture	12.6	Wild Culture	11.6

the laboratory, and (4) there is an average rate for all Paramecia kept under the same physiological conditions. In this case the average for the anterior vacuole is 12.9 and for the posterior vacuole 12.1. These figures are remarkably close to the normal average set by Ehrmann (8) which was 12.

In the experiments with glandular extracts individuals from various wild cultures were used. Since the pulsations do not seem to be subject to specific pure line characteristics it was thought wiser to work with individuals not kept for any length of time in the artificial environment of distilled water and malted milk. It has already been shown that Paramecia taken from a wild culture in spring water, and placed on depression slides with 2 drops of distilled water and 1 drop of 1 per cent. malted milk solution daily show after three and four days of this treatment in a moist chamber at room temperature a perfectly normal rate of pulsation. The following glandular preparations of Parke, Davis & Co. were used—adrenalin in crystal form, pituitary substance made from the desiccated anterior lobe of the pituitary body, and pineal gland tablets prepared from the desiccated gland. Each of these preparations produces a specific effect upon higher organisms, the first contracting the arteries and capillaries, raising the blood pressure, and stimulating the heart, the second acting as a powerful stimulant to growth, and the third slightly increasing the blood pressure. The object of the following experiments is to discover whether these glandular products have an equally specific effect upon the pulse frequency of the contractile vacuole. The preparations were made up with distilled water into solutions of varying strengths, 1–2,000,000, 1–1,000,000 and 1–200,000. Observations were made immediately after adding drops of a solution to the culture medium. The results of the experiments are shown in the tables.

TABLE I.

EFFECT OF ADRENALIN, 1–2,000,000.

Normal Conditions.		1 Drop Adrenalin, 1–2,000,000.	
Ant.	Post.	Ant.	Post.
12.0	12.2	9.8	11.4
9.8	10.2	9.0	11.4
10.2	10.0	10.2	10.0
10.4	9.4	9.0	11.0
10.2	11.0	9.2	11.0
10.1	11.6	10.0	12.2
9.8	12.2	10.0	10.0
10.2	12.2	9.4	9.2
10.2	11.0	9.4	11.0
10.2	11.4	9.8	11.0
103.1	111.2	95.8	108.2
10.3	11.1	9.5	10.8

The increase in pulse frequency after the addition of one drop of adrenalin, 1–2,000,000 is insignificant. No greater increase occurred at any time within two hours after the treatment with adrenalin.

Fig. 1 represents graphically the effect of adrenalin upon the vacuoles of a *Paramecium* in an abnormal physiological condition, produced by some unknown factor. Treatment with one drop of adrenalin, 1–2,000,000 solution did not prevent the rapidly diminishing frequency. One drop of adrenalin, 1–1,000,000 solution produced an immediate quickening in both vacuoles,

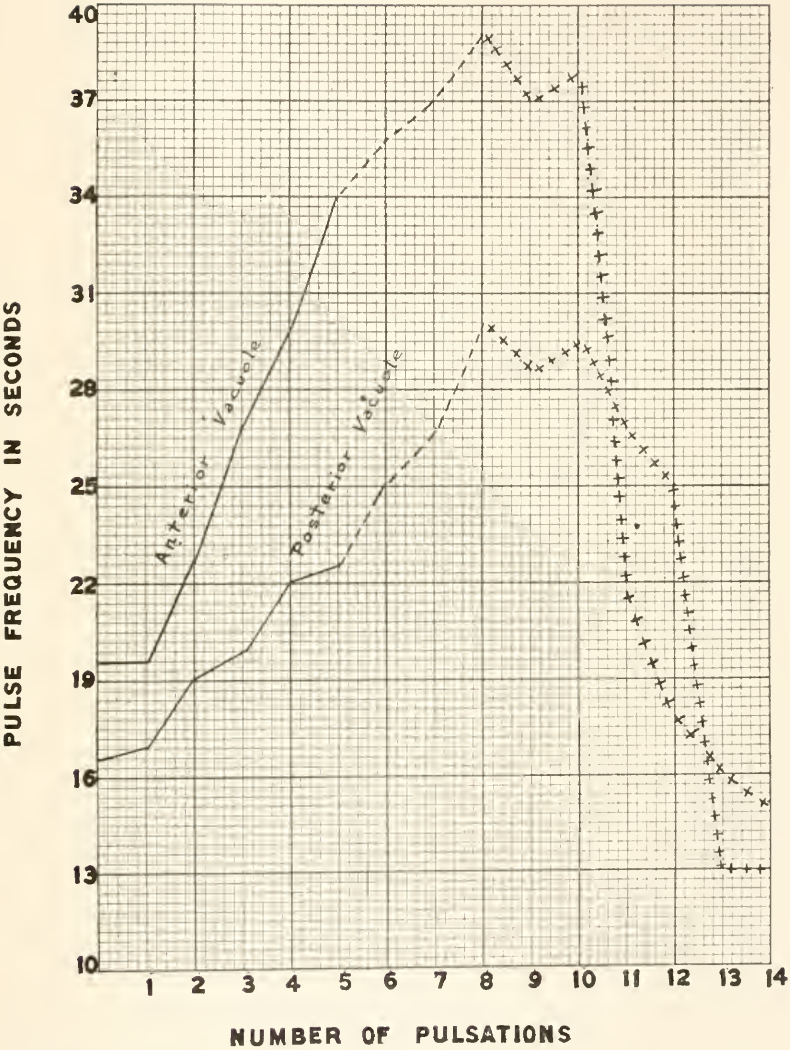

FIG. 1. Increase in pulse frequency following treatment with adrenalin. Ordinates represent pulse frequency in seconds, abscissæ, the number of pulsations recorded. Unbroken lines indicate pulsations previous to treatment with adrenalin. Broken lines indicate pulsations after treatment with 1 drop of adrenalin, 1 : 2,000,000 solution. Lines of crosses indicate pulsations after treatment with 1 drop of adrenalin, 1 : 1,000,000 solution.

continuing until a practically normal rate of pulsation was restored. However, the effect was not lasting. Observations made fifteen minutes later showed a rate of 50 for the anterior, and 42 for the posterior vacuole.

Table II. gives a detailed record of the reaction to adrenalin-1–200,000 solution, in two individuals. From these results and many other similar ones it seems probable that adrenalin of the above strength has a specific quickening effect upon the contractile vacuole. This effect is usually of very short duration, especially if it produces an abnormally rapid rate of pulsation.

TABLE II.

EFFECT OF ADRENALIN, 1 : 200,000.

Normal Conditions.				1 Drop Adrenalin, 1 : 200,000.			
A.		B.		A.		B.	
Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.
13.0	19.0	11.0	12.2	13.2	15.0	10.2	12.0
12.8	18.6	10.0	12.0	11.2	15.0	8.2	11.0
14.4	20.0	12.0	12.0	13.0	14.4	7.6	10.2
16.0	20.2	12.2	12.0	13.2	14.4	8.0	9.2
15.0	20.0	11.2	11.4	13.0	14.0	8.0	9.0
15.2	19.2	9.8	12.0	13.0	14.0	8.2	9.0
14.6	18.8	12.4	12.4	13.0	13.2	8.6	12.8
15.2	20.6	11.0	10.6	13.0	14.0	9.6	13.0
15.0	18.2	9.2	12.2	12.0	13.0	12.0	13.0
15.0	19.6	11.0	11.2	12.2	13.4	12.0	12.0
146.2	194.2	109.8	118.0	125.8	140.4	92.4	111.2
14.6	19.4	10.9	11.8	12.5	14.0	9.2	11.1

Treatment with adrenalin, 1–200,000 solution, produces also a marked dilation of the vacuoles which persists for some time after the rate of contraction has returned to normal. From several records there are indications that the duration of the pulse acceleration is prolonged by increase in the strength of the solution up to a certain point which has not been exactly determined. In three individuals one drop of adrenalin, 1–10,000 solution proved fatal. Not enough records with intermediate strengths have been taken to warrant any definite assumption of a specific optimum strength.

It is evident from the records in Table III. that the effects

Table III.

Effects of Adrenalin on an Individual with Three Vacuoles.

Normal Conditions.			1 Drop Adrenalin, 1 : 200,000.		
Pre-Ant.	Ant.	Post.	Pre-Ant.	Ant.	Post.
19.6	17.0	17.0	18.0	17.0	17.6
20.0	16.2	17.0	17.0	15.0	13.0
20.0	17.2	17.0	19.0	16.0	14.0
18.0	17.0	18.0	18.4	16.4	14.2
21.0	16.8	17.8	19.0	15.2	15.0
18.8	17.0	18.2		14.2	14.0
19.2	16.4	18.2		14.0	
19.6	16.4	17.0		13.8	
20.0	17.0	17.0		14.0	
19.8	17.0	17.8		13.8	
196.0	16.80	175.0	91.4	149.4	87.8
19.6	16.8	17.5	18.2	14.9	14.5

of adrenalin on an individual with three vacuoles do not differ from the effects on an individual with two vacuoles.

While the records in Table IV. do not represent a complete series, there appears to be no very significant increase in pulse frequency as a result of treatment with pituitary, 1–2,000,000 solution.

Table IV.

Effect of Pituitary, 1 : 2,000,000.

Normal Conditions.		1 Drop Pituitary, 1 : 2,000,000.	
Ant.	Post.	Ant.	Post.
18.0	17.2	17.2	15.0
18.4	16.2	17.4	15.0
19.0	16.2	17.4	15.2
18.4	17.2		
17.6	16.8		
18.2	16.2		
109.6	99.8	52.0	45.2
18.2	16.6	17.3	15.0

Table V. gives a detailed record of the reaction to pituitary, 1 : 200,000 solution, in two individuals. From these results and other similar ones it seems probable that pituitary substance of this strength has a quickening effect upon the contractile vacuoles equivalent to the effect produced by adrenalin. In general the accelerated pulse rate is of longer duration under the influence of pituitary substance, but dilation of the vacuole is less marked. Vacuoles contracting at an abnormally slow rate, averages 48 and 42, were not stimulated to a normal rate by the addition of pituitary substance. After being accelerated to 35 and 33 respectively the vacuoles returned to their former abnormal condition. The records taken were not sufficient for the the determination of the lethal or of the optimum solutions.

TABLE V.

EFFECT OF PITUITARY, 1 : 200,000.

Normal Conditions.				1 Drop Pituitary, 1 : 200,000.			
A.		B.		A.		.	
Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.
17.2	16.2	13.6	16.2	17.0	14.6	8.2	15.0
17.0	15.0	12.0	17.0	17.0	15.0	10.0	13.4
19.0	15.0	13.0	18.6	15.0	13.0	9.6	14.4
16.6	15.2	13.2	18.4	14.2	12.8	11.0	14.0
19.0	15.6	15.6	17.8	13.4	13.2	11.2	11.4
21.0	16.2	15.0	17.8	15.4	13.0	9.4	12.8
20.0	16.0	12.4	18.0	16.8	13.6	9.8	11.0
19.0	16.0	13.0	18.0	16.2	13.2	10.2	12.0
18.0	15.8	13.0	17.4	15.2	12.4	10.4	12.0
19.2	16.0	14.0	17.0	15.2	14.2	10.2	12.0
186.0	157.0	134.8	176.2	155.4	135.0	100.0	128.0
18.6	15.7	13.4	17.6	15.5	13.5	10.0	12.8

TABLE VI.

EFFECT OF PINEAL GLAND EXTRACT, 1 : 200,000.

Normal Conditions.				1 Drop Pineal Gland 1-200,000.			
A.		B.		A.		B.	
Ant.	Post.	Ant.	Post.	Ant.	Post.	Ant.	Post.
15.2	16.8	21.2	22.6	12.2	18.0	15.6	13.8
15.0	18.0	20.8	20.8	12.0	15.2	15.8	13.8
15.0	17.0	19.2	22.8	12.4	14.0	14.2	12.8
16.4	17.0	19.6	21.2	12.6	15.0	12.4	13.0
14.2	18.0	18.8	22.0	13.0	15.0	13.2	13.0
14.8	17.0	18.8	20.8	12.2	15.0	13.6	12.0
14.4	18.4	18.8	20.0	12.6	17.0	12.8	12.6
15.0	19.0	19.6	20.6	13.0	16.0	14.6	12.2
15.0	17.2	19.8	22.0	14.0	15.0	14.2	12.8
15.0	16.8	19.0	22.0	13.0	15.0	13.8	13.0
150.0	175.2	195.4	214.8	127.9	155.2	140.2	129.0
15.0	17.5	19.5	21.4	12.7	15.5	14.0	12.9

Table VI. gives a detailed record of the reaction to pineal substance, 1 : 200,000 solution, in two individuals. From these results and other similar ones it seems probable that pineal substance of this strength has a quickening effect upon the contractile vacuoles equivalent to the effect produced by adrenalin and pituitary substance. The pineal substance, however, is much slower in producing acceleration. The results recorded in Table VI. were not observed until an hour after treatment with the solution, when slight dilation of the vacuole also occurred. Vacuoles contracting at an abnormally slow rate, 42 and 38 average showed no significant quickening after treatment with the solution. The records taken were not sufficient for the determination of the lethal or the optimum solutions, but it was noted that the substance in 1–2,000,000 produced no stimulating effects whatever.

Fig. 2 represents graphically the response of Paramecia, with vacuoles contracting at approximately the same rate, to the three different glandular extracts. The average of both vacuoles is shown by each curve. The greatest acceleration is produced by the pituitary substance and the least by the pineal substance.

It is evident from these experiments that solutions of adrenalin, pituitary substance and pineal gland tablets produce an acceleration of pulse frequency in the contractile vacuoles of *Paramecium*. The exact nature of this stimulus is difficult to determine. Each of the solutions employed gave a neutral test, but Rossbach (24) and Degen (6) found that neutral substances produced retardation rather than acceleration. The stimulation cannot be due merely to decreased concentration of the culture medium since the addition of one drop of distilled water to the culture does not produce any marked effect unless the point of evaporation has been almost reached. It seems probable therefore that the stimulation of the vacuoles is produced entirely by the autacoid principles of the glandular extracts. It is questionable, however, whether the stimulation is produced directly by the action of these agents on the vacuole itself, or whether it is the indirect effect of increased metabolism in the entire organism. One is tempted to homologize the effects produced by adrenalin on the vacuoles and on the heart of higher animals, but this seems

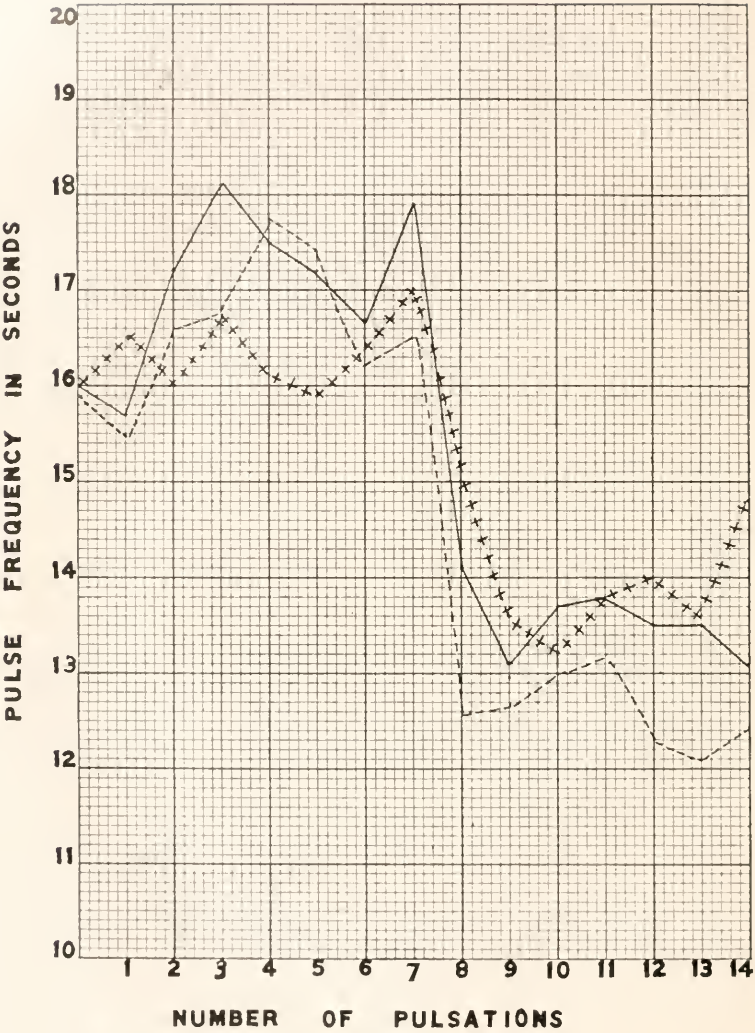

FIG. 2. Increase in pulse frequency following treatment with adrenalin, pituitary substance, and pineal substance, 1 : 200,000 solutions. Ordinates represent pulse frequency in seconds, abscissæ, the number of pulsations recorded. The average of both vacuoles is given. Pulsations 1–7 represent rate under normal conditions, and 7–14 the rate after treatment with the solutions. Unbroken line gives record of individual subjected to adrenalin. Broken line gives record of individual subjected to pituitary substance. Line of crosses gives record of individual subjected to pineal substance.

unwarranted since the presence of a permanent vacuolar membrane is so extremely doubtful. Also it must be noted that pituitary substance appears to produce an even greater quickening of the pulse rate, while in higher animals it has no effect whatever on contractile tissues. Degen (6) it will be remembered claims that the acceleration activated by currents of oxygen is merely a compensation for the increased output of carbon dioxide. A similar explanation seems most plausible for the phenomena produced by the glandular extracts.

Cannon (25) found that inhibition of a strip of intestinal muscle was produced by adrenalin solution as weak as one in twenty mill ons, and Janeway and Park observed inhibition of a strip of coronary artery of the sheep with a solution of one in fifty millions. Takayasu using hemisine, a salt of adrenalin, noted that a solution of two in a million seemed to be the threshold strength for inhibition of the sartorious muscle of the frog. In 1917 Barbour and Spaeth (2) in studying the pharmacological action in single cells found that epinephrin, the active principle of the suprarenal capsule produced contraction of the melanophores of *Fundulus heteroclitus* in all concentrations tested up to one in fifty million. When one considers the far-reaching effects of the various autacoid principles on highly differentiated organisms, it is not improbable that the stimulation of a one-celled organism should be immediately productive of increased metabolism, in compensation for which the contractile vacuoles must become more active in their excretion of waste. The duration of the heightened metabolism will be determined to a certain extent by the concentration of the solution and the neutralizing effect of the digestive fluid. The autacoid principle of adrenalin is believed to be speedily rendered inactive by digestive ferments. To confirm this hypothesis one should find that an increased pulse frequency always occurs with increased division rate. The converse would not necessarily be true, since the metabolic effects might be too temporary to effect the division rate. Schumway (26) noted that under the stimulation of thyroid extract a rapid pulse rate was coincident with a rapid division rate. Much work remains to be done to confirm this hypothesis. Other glandular extracts should be used and individuals in varying physiological conditions should be experimented upon, while especial care should be taken to correlate the different effects produced on the metabolic activity of the entire organism.

The author wishes to express her gratitude to Dr. Florence Peebles for her helpful supervision of the investigations.

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EXPERIMENTS IN BREEDING AS A MEANS OF DETERMINING SOME RELATIONSHIPS AMONG CYCLOPS.

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Two of the largest as well as the most common forms of *Cyclops* are *Cyclops signatus*, var. *coronatus* (*C. fuscus* Jurine) and *Cyclops signatus* var. *tenuicornis*[1] (*C. albidus* Jurine).

Herrick, in his key to the *Cyclops* ("Copepoda, Cladocera and Ostracoda of Minnesota") says of these two forms: "Of the identity of the two varieties which have so long been recognized as distinct species (*C. tenuicornis* and *C. coronatus*) the writer has little doubt. The developmental history has been traced sufficiently to settle this point."

C. tenuicornis and *C. coronatus* are easily distinguishable by the naked eye when adult, and on closer observation they are found to possess several different and very constant characteristics.

C. tenuicornis is generally smaller than *C. coronatus;* the chitin is less deeply pigmented and less hairy; the second segment of the antennule is relatively longer than the corresponding segment in *C. coronatus* as is also the basal joint of the fifth foot.

C. coronatus is larger than *C. tenuicornis;* the chitin is more strongly colored and more hairy; the second joint of the four-jointed antennule is always relatively short as is also the basal joint of the fifth foot. When adult, the female carries her dark egg-masses closely applied to the abdomen. In *C. tenuicornis* the egg-masses are divaricate.

I have attempted to decide the question of any possible identity of these two forms by studies in inheritance.

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[1] *C. signatus annulicornis* seems to be synonymous with *C. signatus tenuicornis*. Fig. 2, Plate XXXIII., Copepoda, Cladocera and Ostracoda of Minnesota, shows the fourth swimming foot of *C. tenuicornis* with two well-formed setae on the inner margin of the distal segment of the inner ramus. The margins themselves are also strongly hairy. These characteristics are constant in all stages of *C. signatus*, var. *coronatus*, but I have not noted them in *C. tenuicornis* (*annulicornis*).

During the study of the metamorphosis of Cyclops, which is about to be published, I have had the opportunity of observing many details of structure throughout their entire history and have been able to compare these details in one Cyclops with corresponding details in another form.

Two of the forms used in the study of the metamorphosis were *C. signatus*, var. *tenuicornis* and *C. signatus* var. *coronatus*. In *C. tenuicornis* the armature of the distal segment of the inner ramus of the fourth swimming feet is very characteristic. The inner margin of this segment carries two setæ, the more distal of which is extremely small or may even be wholly wanting in which case the site of the seta is marked be a notch in the chitin.

In *C. coronatus* this distal seta is fully developed agreeing in size with all the other setæ in the armature.

While the fourth swimming feet are among the later structures to develop during the metamorphosis, they may be said to undergo four stages in development. During the first stage they are too immature to even suggest their later characteristics. During the second stage the appendages are well formed though only one-jointed; Nevertheless, the terminal armature is present and the setæ on the inner margins of the inner ramus already show their adult characteristics. In the young of *C. signatus* var. *tenuicornis* the distal seta is reduced as in the parent form from which the young is derived. When the next molt occurs the foot becomes two-jointed but the distal armature of the distal segments remains unchanged. After the next molt the appendages become three-jointed as in the adult. Still the distal armature is unchanged and though the Cyclops molts again before acquiring its adult condition no further changes occur in the armature of the swimming feet. New setæ and spines may be added as new segments are formed but each seta retains its original characteristic throughout the metamorphosis.

These studies were made on the broods of females that were isolated while they were carrying their appended egg-masses.

Each female was transferred to a separate aquarium—usually to a small *petri* dish. The water was carefully filtered and microscopic algae were added after careful examination under the microscope showed them to be entirely free from any forms that might contaminate the results of the experiments.

The best results in rearing the young were obtained when the water used in the aquaria was the filtered water from the pond in which the cyclops had been collected. After having set up aquaria with water from various sources, this method was relied on as the one which insured least loss of time through high mortality.

EXPLANATION OF PLATES.

Plate I. shows the inner ramus of the fourth swimming foot of an adult female, *C. signatus* var. *tenuicornis* and the corresponding ramus in each of her seven young. The rami are unsegmented and the armature is incomplete, but the distal seta presents its adult characteristics. In four of the sister forms the seta is present but minute. In three of the young it is altogether wanting though its position is indicated by a notch in the chitin.

Fig. 10 of Plate I. shows the inner ramus of another adult female with the small seta. Figs. 11 and 12 show the corresponding rami in two of her brood. At the time the studies were made the remaining young of this set had not reached the stage in which the fourth foot develops.

Plate II. shows the young of a brood in which some young are in the sixth stage, *i. e.*, with nine antennal segments, some in the seventh stage with ten antennal segments and all the rami two-jointed, while still others are in the eighth stage of the metamorphosis having three joints in the rami of the swimming feet. All of the individuals of all of the successive stages show the seta in question to be strikingly reduced.

We are evidently dealing here with a constant character which appears in a relatively early stage of a complicated metamorphosis and retains its peculiarity in all subsequent stages and even remains as one of the recognition features of the adult.

Similar experiments were made in rearing the young of *C. signatus* var. *coronatus* in which all of the setæ attain a marked development.

Plate III. shows the inner ramus of the fourth swimming foot of an adult female *C. signatus* var. *coronatus*. The distal seta is large and well developed. The young are like the female parent in their armature. Moreover, the hairiness of this Cyclops is not an indication of age, but an inherited character for the marginal hairs are strongly developed when the foot first appears, long before these appendages are perfectly developed and before the metamorphosis is complete.

The constancy with which the individual characteristics occur throughout the successive stages of a complicated metamorphosis in both of these Cyclops would seem to indicate that the two forms are not at all identical as Herrick supposed, but distinctly different:

Indeed the reappearance of these same peculiarities generation after generation shows that we are dealing with phenomena of inheritance that have become fully established and not with variations, for the correlated characteristics have become so fixed in *C. signatus* var. *tenuicornis* (*annulicornis*) and *C. signatus* var. *coronatus* that each breeds true to its own type.

EXPLANATION OF PLATE I.

Cyclops signatus var. *tenuicornis*. (*Cyclops signatus* var. *annulicornis; C. albidus* Jurine.)

FIG. 1. The inner ramus of the fourth swimming foot of an adult female, showing the small distal seta.

FIG. 2. The fifth foot of an adult female, showing the characteristic basal joint of the variety *C. signatus tenuicornis*.

FIGS. 3, 4, 5 and 6. The fourth swimming feet of young in the sixth stage of the metamorphosis, showing the characteristic small distal seta.

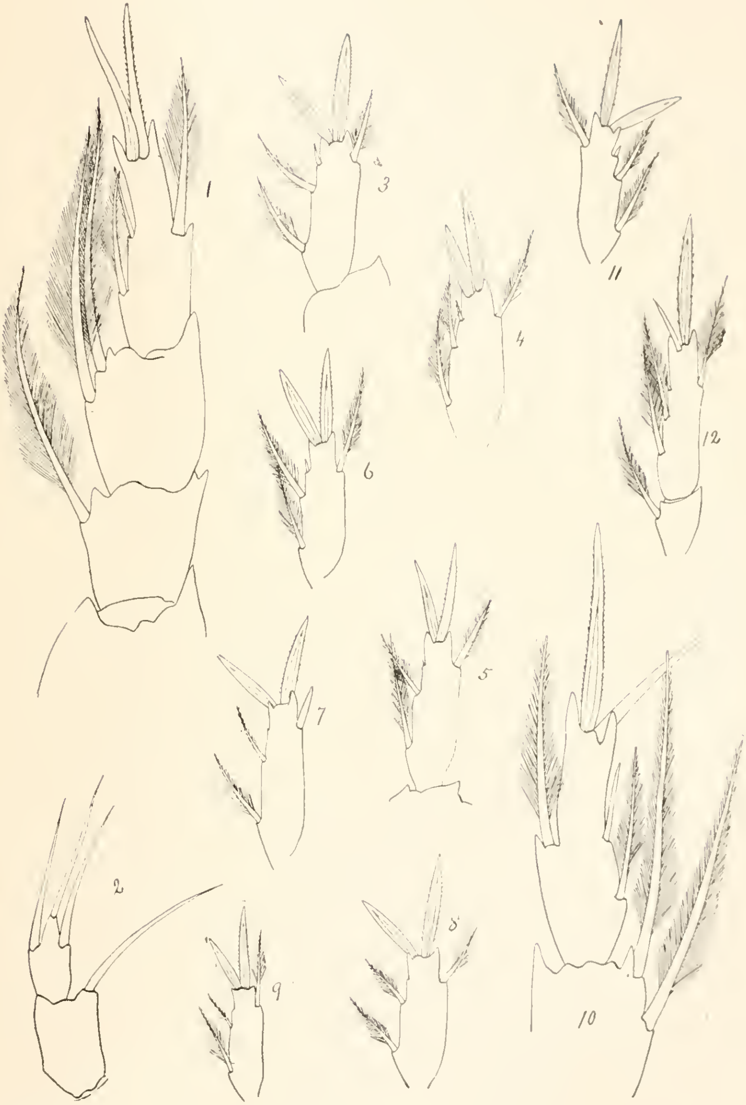
FIGS. 7, 8 and 9. The fourth swimming feet of young *C. tenuicornis*, sister forms of 3, 4, 5 and 6. The distal seta is wholly wanting. The location of the seta is indicated by a notch in the chitin.

FIG. 10. The inner ramus of the fourth swimming foot of an adult female.

FIGS. 11 and 12. Two young of Fig. 10. The sixth stage of the metamorphosis is shown in Fig. 11. Fig. 12 shows the fourth foot in the seventh stage.

The adult female, Fig. 1, was isolated July 28. The young of this female (Figs. 3 to 9 inclusive) were studied August 10. The sixth stage of the metamorphosis was, therefore, reached inside of two weeks.

PLATE I.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EXPLANATION OF PLATE II.

Cyclops signatus var. *tenuicornis.* (*Cyclops signatus* var. *annulicornis; C. albidus* Jurine.)

FIG. 1. The inner ramus of the fourth swimming foot of an adult female, showing the small distal seta on the inner margin of the distal segment.

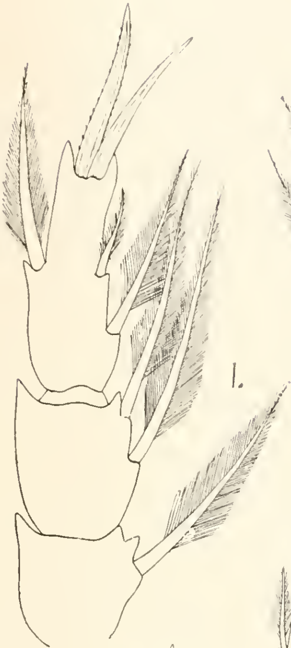
FIGS. 2 and 3. The fourth swimming feet of young *C. tenuicornis* in the sixth stage of the metamorphosis. The ramus is unsegmented. The antennæ are nine-jointed.

FIGS. 4 and 5. The fourth swimming feet of a young *C. tenuicornis* (young of Fig. 1) in the seventh stage of the metamorphosis. The rami are two-jointed and the antennæ *nine*-jointed and *ten*-jointed, respectively.

FIG. 6. The fourth swimming foot of *C. tenuicornis* (young of Fig. 1) in the eighth stage of the metamorphosis when all the rami are three-jointed. The antennæ are *twelve*-jointed.

All the young of Plate II. are sister forms from the same brood. Some are more advanced in development than others, but they are all the same age.

Two-jointed rami are usually correlated with *ten*-jointed antennæ; *three*-jointed rami are usually correlated with *eleven*-jointed antennæ or with *seventeen*-jointed antennæ as in the adult.



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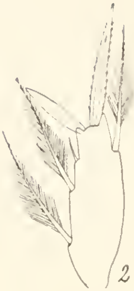

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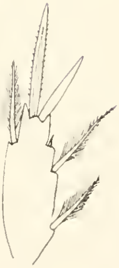

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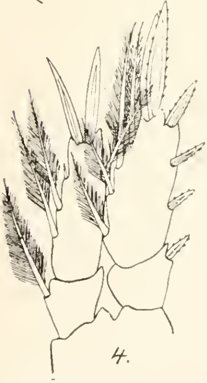

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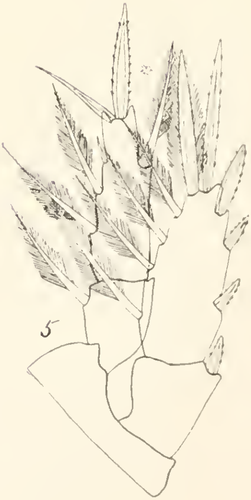

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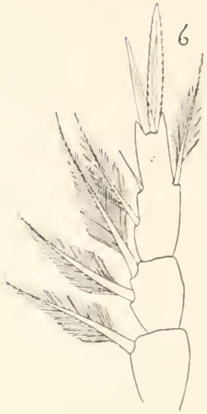

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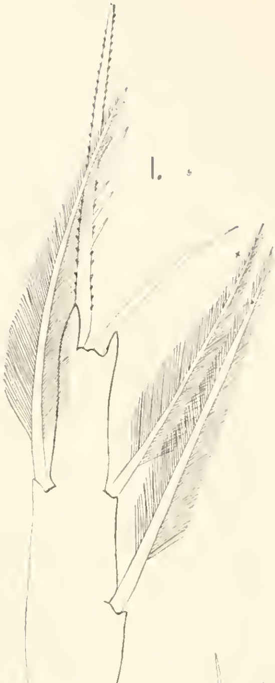
Explanation of Plate III.

Cyclops signatus var. *coronatus*. (*Cyclops fuscus* Jurine.)

Fig. 1. The terminal segment of the inner ramus of the fourth swimming foot of an adult female showing the characteristically large distal seta on the inner margin.

Figs. 2, 3, 4 and 5. The fourth swimming feet of young cyclops in the sixth stage of the metamorphosis, showing the distal seta of the young like the distal seta of the parent.



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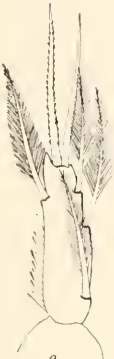

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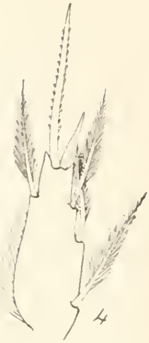

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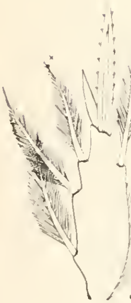

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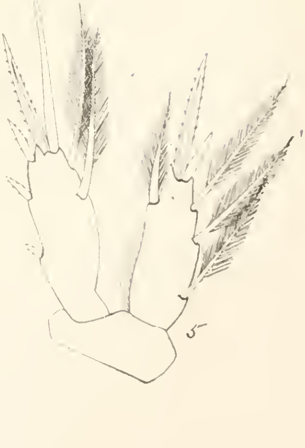

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BIOLOGICAL BULLETIN

STUDIES IN THE LIFE CYCLE OF SIMOCEPHALUS VETULUS.

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I. INTRODUCTION.

These studies on *Simocephalus* were begun at the Hull Zoölogical Laboratory of the University of Chicago, in 1913. The life cycle is very complex and has not been worked out satisfactorily in all of its phases. Although very creditable work has been done by Chambers ('13) on spermatogenesis in *Simocephalus*, and by Weismann and a host of others on the natural history, oogenesis, fertilization, etc., of the Daphnians in general, there is still urgent need of more data. Much time and effort have been expended by the writer in the attempt to perfect a technique which would be adequate to the cytological problems. Considerable progress has been made with this phase of the work yet the study is far from completion. The present paper will be devoted to some problems naturally arising in the course of such studies, which should be solved before or at least in coördination with the cytological problems, else the latter would lose much of their significance.

Among such problems are the following: the normal general life cycle must be known; whether this may not be modified by altering the external conditions and if so, to what extent and how; whether the species may not express itself in all of its forms under each of several sets of conditions, or if there are certain environmental factors which bear special relations to certain forms; whether the failure to produce sexual forms during the parthenogenetic phase is due to a deficiency in the environmental complex or to internal conditions such as age or is dependent simply upon the general rate of metabolism; the normal proportions of males and females; the ratio of sexual[1] to asexual females, the sequence of broods; the cause of senescence of the cultures; the cause of the appearance of the ephippial eggs; the time in the ontogeny at which they appear; the precise function of these eggs, *i.e.*, whether the importance of the ephippial egg is centered in the egg as a means of tiding the species over unfavorable seasons or periods, or in the stem mother developing from it; the relation of the males to the production and development of the ephippial eggs; the normal length of the latent period; if it be of definite duration; whether it can be shortened and if so, how; whether the offspring of a stem mother are different from the offspring of females produced parthenogenetically; whether any difference is to be found between the offspring of parthenogenetic females which have passed through a sexual period and those which have not; whether a sexual female may not be retained in the sexual state, etc.

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[1] By the term "sexual female" in this paper we designate those females producing a series of ephippial eggs which require fertilization; by "asexual female" those which reproduce only parthenogenetically. All so-called sexual females are destined to pass from their sexual phase into the parthenogenetic phase and so remain.

This paper presents data accumulated chiefly during the four years from 1914 to 1918 at Carleton College biological laboratory, Northfield, Minn., bearing upon the solution of these and other problems and definite conclusions regarding some of them. There naturally arises the very interesting and important problem of ascertaining just what correlations exist between the general course and the cytological aspects of the life cycle. The details of my work on this latter phase of the problem are to be given in another paper, now under way.

II. Literature Review.

The theory that there is a sex cycle in Cladocera which is independent of external factors was formulated by August Weismann. It is now most certainly known that, although the correlation existing between environmental factors and the production of parthenogenetic and sexual forms is not exact, certain environmental complexes do exist which completely inhibit the appearance of males and sexual females.

A review of the very extended work of Weismann (1877–87) and his contemporaries such as Kurz (1875) and Schmankewitsch (1877), and of later writers as Issakowitsch (1907), of Kuttner (1909), Woltereck (1909), Papanicolau (1911) and others, leaves one very unsettled as to what is the general determinative principle underlying the course of events in the normal life cycle of even the well known and much studied genera such as *Moina*, *Daphnia*, and *Simocephalus*. The Weismannian conception of a relatively fixed "generation cycle" for *Cladocera* is well known, and has been adopted wholly or in a modified form by practically all writers up to about 1914, although its truth had been questioned by several experimenters. About this time some results were obtained by several workers using various species of the three genera mentioned, which must be interpreted as positive evidence against the theory. Since Weismann's time the recorded opinions range all the way from unconditional acceptance of his extreme view to a complete lack of faith in it. For convenience of discussion these various views may, in general, be grouped under two chief heads:

1. That external factors are of little or of no importance in relation to the succession of periods of parthenogenetic and sexual reproduction. This was the conviction of Weismann, based upon his researches extending over a long period of years. This theory was broad enough to cover all forms of life in which there is a succession of parthenogenetic and sexual reproduction. Accordingly the evidence for the theory extends over a wide field, and it has numerous supporters, having been for a long time the orthodox view.

This general view is supported by Keilhack (1906) and Ekman (1905) working on *Polyphemus*, by Popoff (1907) working on various protozoa and metazoa, and by Punnett (1906) and Whitney (1907) working on *Hydatina*, and by others. The last two authors concluded that the age of the strains is a weighty factor in causing the appearance of the sexual forms. The conclusions of Strohl (1908) constitute a clean cut statement of the views up to that time. Working with *Polyphemus*, he concluded that there was no reason for abandoning the well grounded views of Weismann. Kuttner (1909) working on *Simocephalus vetulus* and other Daphnians adopted Weismann's extreme view. McClendon (1910) considers that "The life cycle of a Daphnid is, therefore an heredity tendency, but can be influenced by nutrition and probably by temperature and the accumulations of excretions," and he adds "Nutrition is the most important factor."

The extended experiments of Woltereck (1909–11) on *Cladocera* led him to admit automaticity as one of the factors in determining the course of their life cycle. Assuming that Woltereck's cultural conditions (1911, fig. 4, p. 152) were kept the same in all four cultures for the full four years, one would be forced to the conclusion that there are periods when environmental factors are of no influence, and possibly other periods when they are, since the four cultures responded not only differently in different years, but different cultures responded differently in the same years; in other words, the conclusion would seem to follow that there is autonomous variation in this respect, which places the whole question beyond the pale of possible experimental proof. The careful experiments of Papanicolau (1911) merit more consideration than is admissible in this paper. The views of Issakowitsch (1907) and Scharfenberg (1911) agree with those of Woltereck and Papanicolau in that they all assume certain inherent tendencies to sexuality which cannot be completely overcome by any kind of environmental conditions.

2. The alternative view is that outer causes, such as chemical substances, hunger, temperature, kind of food, etc., are largely or entirely responsible for the varying degrees of sexual and parthenogenetic reproduction. Evidence for this heterodox view had begun to accumulate at least as early as 1875, when Kurz noted a correlation between the drying up of the water and the appearance of sexual forms in *Cladocera*. Two years later Schmankewitsch (1877) gave as his opinion that the efficient cause is the increasing salt concentration due to evaporation. Among the unfavorable conditions mentioned by Kerhervé (1892, p. 236) poor nourishment is particularly emphasized as being responsible for the appearance of both males and sexual females. Ostwold (1094) found temperature singularly effective as a cause of sexuality in Daphnids. By varying the temperature he produced at the same time all of the forms that are found in nature at different seasons. Langhans (1909, p. 291) says that Weismann's theory of a fixed generation cycle will not bear critical examination.

In regard to other forms whose life cycle is in general similar to that of *Cladocera*, there is much diversity of opinion, although evidence against a fixed internal "cycle" is rapidly accumulating.

Finally we must include under this second category the very significant and definite conclusions of Grosvenor and Smith (1913) working with *Moina rectirostris*, of Banta (1914) working with *Daphnia pulex*, and of Agar (1914) working with *Simocephalus vetulus*, each of whom has conclusively demonstrated that, for the form experimented on, there are certain environmental complexes which will indefinitely inhibit the appearance of males and sexual states in any of the females. It is of interest to note that in some instances the conditions which will thus prevent the full expression of the species is quite narrowly prescribed.

A third category of opinion is sometimes tacitly implied by the manner in which the problem of the succession of forms in *Cladocera* is discussed by those authors who believe that the primary causes are cytological. Were this true the problem would not of course be thereby removed from the domain of possible influence by environmental factors. Here should be mentioned the well-known "kern-plasma" theory of Hertwig. It is supported by Papanicolau, Issakowitsch, Popoff and others.

The results of Von Scharfenberg and Papanicolau have been brought by Child (1915) into relation with certain phases of his general theory of organic constitution, as developed in his book, entitled "Senescence and Rejavenesence." He says (p. 391): "Von Scharfenberg and Papanicolau found that a change in egg character occurred, not only in the course of successive generations, but also in the course of single generations, *i.e.*, the eggs produced early in the life of a female are more likely to develop parthenogenetically into females and those produced later in life into males or to be zygogenic winter eggs. In the earlier generations of a cycle the male producing and zygogenic eggs appear later in the life of the individual, in later generations earlier." I am convinced that the conception that zygogenic eggs normally arise in Daphnians *after* the parthenogenetic eggs is based on entirely insufficient evidence. Definite statements bearing on this point will be found in Papanicolau's papers (1910, p. 740, and 1911, p. 82). These views are in substantial agreement with those of Issakowitsch (1907) and Scharfenberg (1911), and diametrically opposed to my own since I have never found an instance of the production of ephippial eggs following a period of parthenogenetic reproduction. I am convinced that these conclusions could not have been based upon observations on isolated individual females of *Simocephalus vetulus*. Grosvenor and Smith (1913, p. 514) working on *Moina rectirostris* state: "We did not find any case of a female that had produced eggs parthenogenetically turning into an ephippial female."

For several years the writer was not able to duplicate the results of those experimenters who claimed that, under certain conditions, reproduction in some Cladocera will proceed for an indefinite number of generations parthenogenetically, and that the change to sexual reproduction, together with the degeneration that so often accompanies it under experimental conditions, are wholly dependent upon the environment. Later experimentation however (experiment 5), has demonstrated their contention to be correct, at least for *Simocephalus vetulus*. Though these last experiments are of a very different type from those described by Agar (1914*b*, Table I.), Grosvenor and Smith (1913), and Banta (1914), who succeeded in carrying Daphnians through many generations with no loss of vigor, they are corroborative of their results. That the success of these authors in rearing purely parthenogenetic generations for an indefinite time could not have been due to the accidental selection of lines with a strong internal tendency to parthenogenesis is rendered certain not only by the use of several distinct lines, but also by parallel cultures subjected separately to unfavorable temperatures and crowding, which gave a large proportion of males and sexual females. The genera used were *Simocephalus*, *Moina* and *Daphnia*. Banta carried *Daphnia pulex* for 127 generations with no loss of vigor.

In groups other than Cladocera in which it has been found that an indefinite number of generations can be reared parthenogenetically, it seems that it has always been possible to bring on sexuality by a proper change in the environment, which would indicate that pure lines with respect to parthenogenesis do not exist. It now seems altogether likely that bisexuality may be indefinitely inhibited in many more of the lower animals than experimenters have been aware of heretofore. The conditions under which this is possible have been found in some instances to be narrowly circumscribed, as *e.g.*, certain Cladocera require the high temperature of 28° C., although the more recent authors seem to believe that a considerable number of species of Cladocera will be found which can be induced to reproduce by parthenogenesis indefinitely if only the external conditions are properly manipulated. It appears also that, whatever may be the explanation, any set of nearly uniform conditions finally becomes prejudicial to continued parthenogenesis. Parthenogenetic reproduction seems to be favored by conditions which are conducive to rapid growth, though they may vary within certain limits.

Each species of Cladocera found capable of pure parthenogenesis has its own specific requirements as to which environmental factors may vary and to what extent without it becoming zygogenic.

III. MATERIAL.

On taking up this work much difficulty was encountered in rearing algæ as food for the Daphnians. Numerous formulæ were tried with varying success, but for some reason they did not thrive on algæ artificially produced. A simple and satisfactory solution of the difficulty was discovered in the following method: A number of three- or four-gallon aquaria nearly full of water are placed where they will not receive the direct rays of the sun and stocked with several kinds of unicellular algæ. One to three frogs, depending upon the size, which have been in captivity until little or nothing remains in the alimentary canal, are placed in each of the jars. Of course the aquaria must be covered, in part to retain the frogs, but the more important reason for this is to insure the maintenance of a high percentage of carbonic acid gas and the depletion of oxygen. Under such conditions the algæ grow rapidly until a condition of equilibrium is reached. When a sufficient quantity of algæ has developed a single large Daphnian with brood pouch full of eggs or embryos may be introduced and the frogs removed, or one may be left in with good results. When the Daphnians have overstocked the culture they may be strained out with a cloth and transferred to a new jar. The brown sediment of the old culture, which is excreta and dead algæ, should be carefully removed so as not to loosen the algæ on the sides of the jar, new water added and more algæ grown in the manner indicated above.

The above method has served me very satisfactorily for *Simocephalus vetulus*, though it is not so well adapted to some other forms. *Daphnia pulex* thrives in water which is more or less filled with putrescent matter. In a pond frequented daily by cattle and thus kept very roily and malodorous, I found this species in such numbers that by a single dip of the net I secured hundreds of them. For best results one needs to work out special methods for each species. In all of my work I have used *Simocephalus vetulus* unless otherwise stated.

IV. Isolation Experiments.

In order to ascertain the course of events in the normal general life cycle I isolated numerous specimens, at random at first, and kept a complete record of all of their offspring. Some of these will be presented in detail. For convenience all experiments will be referred to by number.

Experiment 1.—On June 23 a very large female with the brood pouch full of embryos was isolated. On June 24 a brood of 47 asexual females was extruded. These embryos were saved and a first brood secured from each of 25 of them. The remainder died early. There were 13 pure broods of males, the numbers in the broods being as follows: 4, 3, 1, 3, 1, 1, 1, 2, 2, 2, 6, 2, and 7. There were 6 mixed broods as follows: 3 males and 6 females, 8 males and 4 females, 6 males and 6 females, 1 male and 6 females, 5 males and 3 females, and 2 males and 4 females. The numbers of females in the pure broods of females were: 4, 5, 6, 3, 4 and 2. Now with this single instance before us we might well ask why this great variety in the offspring of the members of a pure brood of 47 asexual females? But in the light of further experimentation along this same line we may reasonably suppose that had the mother of these 47 female embryos lived to produce other broods, some would have consisted of males or at least would have contained males.

Another female isolated at the same time lived until July 13, when she died with embryos in her brood pouch. Her series of broods is as follows: 1 male and 6 undetermined, 7 females, 15 males, 50 females and 1 male, 15 males, 9 females, and 13 females. The 50 females of the mixed brood were saved and the first brood of each of 19 of them was secured. There were 12 broods of males containing 8, 12, 3, 8, 12, 3, 12, 4, 5, 41, 12 and 6. There was one mixed brood of 12 males and females, and 6 pure broods of females contained 6, 10, 1, 3, 2 and 6.

There seemed to be no constancy as to the ratio of males to females in the offspring of these isolated females, nor in the sequence of broods, some producing males first, some females, and others mixed broods, in a most capricious manner. Hence these miscellaneous experiments demonstrated the desirability of much more extended experimentation to discover if possible what order might underlie this apparent confusion.

Experiment 2.—The following experiment was designed to show in particular the normal ratio of males to females, and their sequence, though it furnishes data bearing upon several other points. It was begun on June 23 by the isolation of a single female from a laboratory culture. She produced the following broods on the dates indicated·

On June 26 a brood of 43, 27 being female, 16 not living.

On June 28 a brood of 49 females.

On July 3 a brood of 75 females.

On July 7 a brood of 7 males.

On July 10 a brood of 6 females.

On July 11 the isolated female died.

Of the brood produced on June 28, 45 which lived were saved for experiment. These were isolated and the sex of all of their offspring determined, and in several instances the kind of females *i.e.*, whether sexual or asexual. The members of this brood are numbered. The date of the first broods of each of the first 17 was July 4, of all of the remainder it was July 5. The date of the death of each female is given at the end of her series of broods. The date of the last brood is practically always not more than one day before the death of the female. The second brood of female number 1 is a mixed brood and they are so indicated throughout.

1. 9 females, 5 females and 9 males, 9 females, an undetermined brood, July 15.

2. 9 females, a mixed brood of about 20 containing many dead, 6 females, July 12.

3. 4 females, 3 males and 6 undetermined, 11 males, 4 females, July 13.

4. 4 females, 18 males and 2 females, 6 females, 1 female, July 13.

5. 3 females, 11 males and 8 females, 16 females, July 9.

6. 9 males, 3 undetermined, 6 females, 22 females, July 10.

7. 7 females, 7 females and 8 undetermined, 6 females, 5 females, an undetermined brood, July 14.

8. 6 females, 3 females and 10 undetermined, 10 males, July 7, 14.

9. 8 females, 2 males, 4 males, July 9.

10. 6 females, 3 males and 16 undetermined, July 7.

11. 6 males and 1 undetermined, 4 males and 1 female, 4 females, an undetermined brood, July 13.

12. 8 males, 3 males, 3 females, an undetermined brood, July 9.

13. 7 males, 1 male, 7 females, July 9.

14. 9 females, a mixed brood undetermined, 6 females, July 12.

15. 6 females, 9 males, 7 females, July 8.

16. 10 males, 6 males, 5 females and 4 undetermined, 10 females, July 9.

17. 9 females, 38 females, 10 males, July 15.

18. 12 females, 7 females and 6 males, 10 undetermined, 7 females, July 9. All of the last brood of 7 females were asexual but some of their offspring were sexual.

19. 6 females, 10 males and 14 undetermined, 7 females, 14 females, July 9.

20. 8 females, 21 males, 8 females, two other undetermined broods, July 10.

21. An undetermined brood, 11 males and 12 females and 14 undetermined, July 9.

22. 7 females, 12 males and 7 females, an undetermined brood, July 9.

23. 10 males, 2 males and 13 females, 3 females, July 13.

24. 4 males, 6 asexual females, 10 females, 2 sexual females, 15 males, 24 females of which 2 were sexual and 2 asexual and 8 males, (the other 20 females of the last brood died too early for their sexuality to be determined), 13 females 7 of which were asexual and 6 undetermined, 16 asexual females and 1 male, 2 females 1 being asexual and 1 sexual, 22 females 12 being sexual and 10 undetermined, the next two broods were accidentally left together but their sum was 35 females of which 12 were sexual and 11 asexual while the remaining 12 were undetermined, the final brood was 7 females of which 5 were sexual 1 asexual and 1 undetermined, August 2.

25. 5 females, 20 males, 4 females, July 9.

26. One male and 5 females, 11 males and 2 females, 7 females, July 10.

27. 4 males, 1 male and 4 females, July 7.

28. 7 females, 12 males, 3 sexual females, 7 males, 1 male, July 12.

29. 5 males, 7 males, 6 males, 2 males and 11 females, July 14.

30. 5 females, 8 males and 9 undetermined, July 8.

31. 4 males, 8 males, July 7.

32. 8 females, 16 males, 1 male, July 9.

33. 2 females, 22 males, 8 females, July 9.

34. 6 males, 7 males, July 8.

35. 6 males, 5 males, July 8.

36. 8 males, 5 males, 4 females, July 9.

37. 8 females, 7 males, July 8.

38. 5 females, 22 females, 6 males, an undetermined brood, 20 females, 1 male and 35 females, July 15.

39. 19 males, an undetermined brood, 15 females, July 8.

40. 4 females, July 7. (mother with abnormal carapace, offspring normal).

41. 3 males, 3 males, 6 females, 7 males and 7 sexual females, 5 males and 2 sexual females, 1 sexual female, 1 female and 5 undetermined, 20 females 15 of which are asexual the other 5 may be, 15 females, 17 females, 3 females 2 of which are sexual and 1 asexual, 2 females 1 of which is asexual the other undetermined, 9 females 7 of which are asexual 1 sexual and 1 undetermined, July 31.

42. 6 males, 2 males, 13 females, 1 male and 2 females, July 13.

43. 5 males and 2 females, 11 males, 7 females, 11 males, 1 male, 6 females, 11 males and 1 female, 2 males, a brood of females, 5 females, an undetermined brood, 1 female, July 26.

44. 7 males, 8 males, 6 males, 2 males, 10 females, 9 females, 24 undetermined, 9 females, 8 females, 12 females, an undetermined brood, 4 females, 4 asexual females, August 4.

45. 8 females, 23 males, an undetermined brood, 8 males, 8 males, 15 males, 10 females, 18 females, 1 male and 2 females, 1 sexual and 1 asexual, an undetermined brood, 7 females, July 24.

Summary of Experiment 2.

The number dying without offspring	4
" " possibly producing no males	3
" " " " " females	2

The percentage of males is about	42
The number of times the first brood was purely female	26
“ “ “ “ “ “ “ “ “ male	16
“ “ “ “ “ “ “ “ a mixed brood	2
“ “ “ “ “ “ “ “ undetermined	1
“ “ “ “ “ last “ “ purely female	28
“ “ “ “ “ “ “ “ “ male	11
“ “ “ “ “ “ “ a mixed brood	6
The total number of mixed broods	22
The number producing no mixed broods	25
“ “ of times the broods immediately preceding and following mixed broods are of the same sex	9
The number of times they are of a different sex	4
The average number in each pure first brood of females	6.6
“ “ “ “ “ “ “ “ “ males	7
“ “ “ “ “ mixed brood	16
“ “ “ of offspring per individual	39
“ “ “ “ broods “ “	4.3
“ “ “ “ hours between the broods	45.3
“ “ “ “ female broods per individual	2.1
“ “ “ “ male “ “ “	1.3
“ “ “ “ females in each female brood	4.5
“ “ “ “ males “ “ male “	11.9
“ “ “ “ “ produced per individual	15.5
“ “ “ “ females “ “ “	20.7
“ “ “ “ days each of the 45 females lived	14
The total number of offspring was slightly over	1705

The 45 females under consideration in this experiment were divided into three groups. The first group, consisting of 24 individuals, was kept in water taken from an "asexual" culture and were well fed; the second group of 20 were also well fed but were kept in water taken from a culture which was producing an abundance of males and sexual females; the third group of 5 being reared in the same kind of water as the second but were poorly fed. The results of this part of the experiment are given in the following table in which the data have been reduced

	Group 1	Group 2	Group 3
Average length of life in days	12.4	11.0	27.2
“ number of broods	3.6	3.0	10.6
“ “ “ female broods	2.1	1.1	5.2
“ “ “ females produced	21.6	12.3	43.4
“ “ “ males “	11.5	15.6	29.2
“ “ “ male broods	.9	1.4	3.2
“ “ “ offspring	37.6	26.6	80.6
	%	%	%
Mixed broods	9.	10	11

to the basis of the individual, the data on the first, second, and third groups being given in the first, second, and third columns respectively, of the table.

Comparing these three groups we find that the differences when averaged are not great enough to be significant. The members of the third group lived roughly two and one half times as long as the others, and should we reduce the data to a common unit of time the correspondence would be very close. For instance, the number of broods produced by the first group per unit of time, say 27 days, would be 10 (2.25 × 3.6 = 10.00), while the third group produces just 10.6. The number of female broods produced by the first group would be 4.72 (2.25 × 2.1 = 4.72), as against 5.2 broods produced by the third group, etc. As to all of the main points under consideration the figures agree so closely that we must conclude that the kind of water in which the three groups were reared and the relative food supply had little or nothing to do with the ratio of sexes. In the general discussion of this experiment further data are given based on the observation of isolated females whose ancestry is now known for one or two generations, with conclusions regarding the relation of sexual to asexual females, males to ephippial egg production, etc. It is deemed better however, to defer this until after the presentation of another general isolation experiment in which stem mothers were used instead of females selected from the general cultures.

Experiment 3.—Although most of my experiments to induce ephippial eggs to hatch have been failures I have given them in detail further on in this paper because of the fact that so little has been accomplished along this line. I have succeeded in securing about 70 stem mothers and have had a fair degree of success in rearing them. All of these were isolated at once and a complete record was kept of the number of broods, the kinds of individuals in each brood, length of life, etc., as shown in the table below. It was found that a large number died early, producing few or no offspring. A few however produced as many as 9 broods. The average number of broods produced per stem mother was slightly over 6. Hence in the tabulation of results no account was taken of stem mothers whose broods were too few, since these were probably not perfectly normal, else they would have lived longer, nor of broods beyond the sixth except to consider them in determining the average number of broods per stem mother, since the averages were not materially changed by eliminating them. Accordingly only 24 stem mothers were considered fit to include in the final averages, and for similar reasons the number of mothers in the F_2 and F_3 generations were reduced to 16 and 12 respectively. I was much surprised to note the large proportions of males and sexual females in the broods of these stem mothers. This observation suggested the experiment of rearing several generations to discover the relative proportions of males and sexual females in the first and later generations, to compare the offspring of the stem mothers with that of the females produced parthenogenetically, and to learn what difference, if any, is to be found between the offspring of sexual females after they have passed through the sexual state and of those females which never pass through this state. It seemed that even if passing through the sexual state did not affect the ratio of the sexes, or of asexual to sexual females, in the immediate offspring, that the effect might conceivably be cumulative and would be apparent in the succeeding generations. Hence the sexual and asexual females in the F_1 generation were segregated and two distinct lines, one sexual and the other asexual, were carried to the F_3 generation. Perhaps the most conspicuous feature in the records of this experiment is one shown only by the individual records of the females, namely that they are variable in the extreme. A given stem mother may produce nearly all males, or one kind of females, for several broods, or throughout her life, or they may appear combined in all proportions, just as is shown in the detailed individual records given in experiment 2, female number 41. The most important facts however are to be deduced from the summary given at the end of the tabulated results. In order to facilitate the comparison of results in the sexual and asexual lines in the second and third generations, I have placed the figures in juxtaposition and have reduced them to a percentage basis at the end of the table, besides giving the chief data in simpler form following the main summary of this experiment. Of course the results in all cases are somewhat in error because of the number of offspring which die too early for identification; but the error is not considerable since all of the available evidence points to the conclusion that all of the different kinds of offspring are about equally viable. Inspection of this table shows no marked changes in the relative number of kinds of offspring from the first to the later broods in any of the generations in either the sexual or the asexual lines. This would corroborate the conclusion already reached from the numerous other experiments, namely that sex is in no way correlated with the age of the mother. It was of the greatest interest to find that the offspring of the F_3 generation in the sexual line were no more likely to be males and sexual females than were the offspring of the F_3 generation in the asexual line. It seems that if such selection were to have any definite effect that it would at least begin to show by the third generation. Yet it will be observed that in the second generation 33.3 per cent. of the offspring in the asexual line is male, and 32.7 per cent. is male in the sexual line; while in the third generation the corresponding percentages are 35 per cent. and 31.5 per cent. respectively. Of the stem mothers the percentage of males in the offspring is 23.5 per cent., the lowest of all. It seems to me however that the differences in no case are great enough to be of any significance in a species of animals which displays so much variation in so many respects as this one does.

The ratio of the asexual to the sexual females is seen to be 27.6 per cent.: 23.1 per cent. in the F_1 generation; 19.1 per cent.: 15.3 per cent. in the F_2 generation of the asexual line, 21.9 per cent.: 11.3 per cent. in the F_2 generation of the sexual line; 20.7 per cent.: 17.6 per cent. in the F_3 generation of the asexual line, and 28.2 per cent.: 12 per cent. in the F_3 generation of the sexual line. The last ratio is striking as showing such a low percentage of sexual females in the sexual as compared to the asexual lines in the same generation.

It will be seen that if the percentages of the males in these five categories are averaged we get somewhat over 31 per cent., which is very much lower than that observed in experiment 2, which was 42 per cent. Since in the latter case the offspring considered numbered over 1,700, it may seem that we should be justified in concluding that the percentage of males in the generations immediately following the stem mothers is normally low. Such a conclusion would be erroneous, since several experimenters have succeeded in maintaining cultures of *Simocephalus* and other genera indefinitely in the parthenogenetic phase. In all cases in my experiments where females were isolated and kept in small containers in the laboratory some of their broods were sure to contain sexual forms if the mothers lived to produce a reasonable number. In the experiment under discussion the sexual forms did not increase up to the sixth brood, after which so large a proportion of stem mothers died that it seemed best to close the experiment. However at this point experiment 5 is complementary since it is concerned with a large number of females which are of varying degrees of remoteness from stem mothers and we find them producing only about five per cent. of sexual forms. It seems quite clear that the remoteness of the generation from the stem mother bears no definite relation to the numerical ratio of male to female offspring or of sexual to asexual females.

As to the degree of sexuality of the sexual females concerned in this experiment I find no evidence that it is different from that observed in the former experiment. The number of ephippia produced can doubtless be taken as a crude index to the degree of sexuality of a female, since, as shown elsewhere, the number produced is dependent upon factors inherent in the female, fertilization of the ephippial egg having no relation whatever to the continuance of the sexual state. The number of ephippia cast by each sexual female in this experiment averages slightly less than two. I have found very few females which produce as many as five ephippia. Only three instances are recorded in all of my experiments. The production of three is common. Here as in all other cases observed, the sexual individuals passed the sexual state by the time they were about half to two thirds grown, and never returned to it once they had become asexual. The only observer so far as I am aware recording the contrary for *Simocephalus vetulus* is Issakòwitsch (1908). Moreover, ephippia which were barely noticeable would sometimes appear and be cast, asexuality coming on at once. Other individuals would develop their ephippia to half or two thirds the normal size and

TABULATED SUMMARY OF EXPERIMENT 3.

Description of the Five Categories.	Offspring of the Stem Mothers, *i. e.*, the F1 Generation.						The F2 Generation. Asex. Line is Upper Figures. Sex. Line is Lower Figures.						The F1 Generation. Asex. Line is Upper Figures. Sex. Line is Lower Figures.					
Kinds of Offspring in these Categories so Far as Known.	Totals.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex not Known.	Totals.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex not Known.	Totals.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex not Known.
Number of mothers	24						16						12					
							16						12					
Average number of broods from each	6.4						6.0						5.5					
							8.2						6.2					
Average time of bearing, in days	28.1						23						19					
							31						20					
Average length of life, in days	36.1						30						29					
							39						32					
Average number of kinds of offspring	15.7	4.2	7.6	.6	1	2.3	10.3	.6	0	6	1.5	2.2	7	1	2	4	0	0
of 1st broods							6.2	1.6	.1	1.5	2	1	9.7	2	.9	3.8	.4	2.6
Average number of kinds of offspring	14.1	2.2	5.1	2.1	1.8	2.9	8.5	3	2.1	1.4	0	2	5	1.5	1	2	.5	0
of 2d broods							5.5	1.7	1.8	.7	1.3	0	8.1	3	0	2.2	1	1.9
Average number of kinds of offspring	9.4	1.6	1.7	3.1	1	2	11.1	2.1	2.3	3.1	2.5	1.1	8	2.1	1.8	1.1	2	1
of 3d broods							8.7	1.3	.4	4	3	0	6.5	.6	1.2	1.7	3	0
Average number of kinds of offspring	12.4	6.2	1	4.5	.4	.3	4.7	1.6	.3	2.1	.7	0	7.5	2.1	.9	2.5	2	0
of 4th broods							6.9	.7	0	2.2	4	0	11	5	2	2.1	1.9	0
Average number of kinds of offspring	11.8	4.5	.8	4.3	1.7	.5	6.6	1.2	.3	2	1	2.1	4.7	.5	1.3	1.9	0	1
of 5th broods							7.5	1	2	3	1.5	0	12.2	4.2	1	4	2	1
Average number of kinds of offspring	7.1	1	.1	1	5	0	5.6	.5	2.2	1	1.6	.3	7	.9	0	2.1	4	0
of 6th broods							4	2	0	2	0	0	12.1	2	2	5	3.1	0
Sum of the six broods	70.5	19.7	16.3	16.6	10.9	8	46.8	9	7.2	15.6	7.3	7.7	39.2	8.1	7	13.6	8.5	2
							37.8	8.3	4.3	12.4	11.8	1	59.6	16.8	7.1	18.8	11.4	5.5
Percentage of females in all six	66.1						50						59.9					
broods							64.4						59.3					
Average number of kinds of offspring	11.7	3.2	2.7	2.7	1.8	1.3	7.8	1.5	1.2	2.6	1.2	1.3	6.5	1.3	1.1	2.2	1.4	.3
of all broods							6.3	1.4	.7	2	2	.2	9.9	2.8	1.2	3.1	1.9	.9
Totals reduced to a percentage basis	100	27.6	23.1	23.5	15.4	11.3	100	19.1	15.3	33.3	15.6	16.1	100	20.7	17.6	35	21.6	5.1
							100	21.9	11.3	32.7	31.2	2.6	100	28.2	12	31.5	19.1	9.2

become asexual. After the production of several ephippia a partial one may be developed as the female leaves the sexual state, or she may change over abruptly. The degrees of sexuality are infinite, since it is subject to continuous and not to integral variation.

Considering the stem mothers from the standpoint of size attained, viability, length of life, activity, etc., in the first few generations of offspring, in addition to the proportion of the kinds of offspring as to sex, and the degree of sexuality of the females, one is forced to conclude that stem mothers are not functionally at all unlike the females which are produced parthenogenetically, with the exception that not a single instance of the production of an ephippial egg by a stem mother has been noted. This last is in strict accordance with the findings of Grosvenor and Smith (1913) for the stem mothers of *Moina rectirostris*, and of most writers, though rarely instances of the contrary are noted, *e.g.*, Scharfenberg (1911, p. 24).

The most important data are excerpted from the tabulated summary of experiment 3 and slightly rearranged in the following table for the convenience of the reader.

Kinds of Offspring.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex Not Known.
Percentage of kinds of offspring (F_1 gen.) in the first 6 broods of 24 stem mothers.....	27.6	23.1	23.5	15.4	11.3
Percentage of kinds of offspring (F_2 gen.) in the first 6 broods of 16 F_1 asexual females	19.1	15.3	33.3	15.6	16.1
Percentage of kinds of offspring (F_2 gen.) in the first 6 broods of 16 F_1 sexual females after becoming parthenogenetic..........	21.9	11.3	32.7	31.2	2.6
Percentage of kinds of offspring (F_3 gen.) in the first 6 broods of 12 F_2 asexual females	20.7	17.6	35	21.6	5.1
Percentage of kinds of offspring (F_3 gen.) in the first 6 broods of 12 F_2 sexual females after becoming parthenogenetic..........	28.2	12	31.5	19.1	9.2

Experiment 4. Temperature experiments with *Simocephalus* yield very indefinite results. Individuals do not thrive at a higher temperature than 28° C. Even at this temperature isolated individuals live only a short time. Several experiments designed to test the effect of high temperature were performed in the following manner. Specimens were taken from laboratory cultures and placed in glass containers having loose covers to

prevent excessive evaporation. In each of these experiments four containers were used, of about one pint capacity, two thirds full of water at room temperature when the Daphnia were introduced. Each vessel contained a sufficient quantity of green algæ for 25 females. These containers were placed in an electric parafin bath and the temperature maintained at 28° C. The first one or two broods produced under high temperatures were found to contain the usual proportions of males and the two kinds of females (see tabulated summary of experiment 3). These first broods were discarded. Thus all of the offspring in the tabulated results of one of these experiments given below, passed their entire ontogeny at a temperature of 28° C.

Dates on Which Offspring were Removed.	Total Number of Mothers Living on Dates Indicated.	Offspring.				
		Asexual Females.	Sexual Females.	Males.	Other Females.	Sex Not Known.
August 25	106	21	0	28	16	3
August 30	102	14	12	30	42	17
September 5	81	6	10	11	38	8
September 7	17	2	0	7	5	8
September 10	3	0	3	2	0	4
September 11	0					
Total number of kinds of offspring		43	25	78	101	41
Percentages		14.9	8.7	27.0	34.0	14.0
Total number of offspring						288

The most important point to be noted in these results is that all kinds of offspring continued to appear to the last. Although the percentages vary somewhat, they are within the limit of normal variation. Other experiments were performed to determine the effect of lower temperatures. All of the kinds of offspring were secured at a temperature of 14° C., but the general metabolism is so much lowered at this temperature that the individuals were too few to justify any conclusions as to the ratio of the sexes. Both low and high temperatures, since they lower the rate of metabolism, induce a decrease in the average size of the broods, in the number of broods, viability of the offspring, and shorten the lives of the females used in experimenting. In this connection it is of interest to note that females which were reproducing parthenogenetically have been obtained every month in the year from shallow ponds near Gary, Ind. The offspring obtained in the winter months were asexual females. While no sexual females and males were obtained from eggs and embryos borne by females at the time of collecting from under the ice, the offspring thus secured were not sufficiently numerous to justify any statement as to the production of males and sexual females at this season. I am uncertain as to just how far these experimental results hold true in nature, but there seems to be no reason for considering temperature a vital factor in relation to the sex cycle of *Simocephalus vetulus*. As mentioned elsewhere, Grosvenor and Smith (1913) completely inhibited sexual forms in *Moina rectirostris* at a temperature of 28° C., but since they could not decide as to what factor their success was due it is probably safe to assume that they succeeded in spite of the high temperature, not because of it.

Experiment 5.—The following experiment was performed to discover what kinds of offspring are produced under natural conditions. It involves 51 females collected at various times from April 20 to May 25, in the vicinity of Northfield, Minn., from three widely separated permanent freshwater ponds. The sizes of the females ranged from small (hence young) to very large (hence old) individuals. The largest female among the lot was slightly over 4 mm. from the anterior margin of the head to the posterior end of the carapace and $2\frac{5}{6}$ mm. in vertical measurement. There is no doubt that the age and size of *Simocephalus* correspond very closely. Though I know the pedigree and brood records of none of these 51 females it may be assumed on the basis of size that some, such as the smallest, had produced very few broods, while others, the larger ones, had produced very many, probably 15 or 20. Although the individuals were not isolated there was no crowding. They were placed in two-quart fruit jars, no jar containing more than six individuals. Jars containing several specimens were kept full. They were placed outside the laboratory windows where the sun would not strike them, being thus subject to the ever varying temperatures. Only water brought from the ponds where the females were collected was used. This was strained through a fine silk cloth which removed all metazoa, but allowed the passage of small unicellular algæ. After the first few days a gradually thickening film of green algæ grew on the sides of the jars. To insure that slight accumulations of excreta and other waste might not affect them the water was changed every two days. No food was provided other than that which was suspended in the water and which passed through the fine strainer. They were thus subjected to the same culture medium, food and the same night and day temperatures, as they were in the natural environment. It was thus hoped to duplicate as nearly as possible the natural conditions of these ponds and to acquire positive evidence as to the kind of offspring being produced under natural conditions by testing only the first broods produced after the females were collected. It will be noted that the tabulated presentation of this experiment gives the dates of the collecting trips in the first column, the number of specimens secured on each trip in the second column, the dates on which all young were separated from their mothers and placed in other similar jars in the third column, the number of offspring removed on each date in the fourth, the number of offspring which proved to be female in the fifth, etc., Records were kept of more than one brood from the females collected on May 17, May 20 and May 25, and it is interesting to note that the fourth brood produced by the single female collected on May 17 consisted of 50 males and one sexual female. In calculating the percentages of kinds of offspring only those first removed were taken into consideration. Of the 524 offspring in this category none is positively known to be male and 98.8 per cent. are positively determined to consist of females. All of the first offspring of the 19 females collected on April 20, May 16 and May 23 were saved to test for sexuality. It is seen that 95.6 per cent. were asexual, unless possibly there were more than 7 sexual females in the brood of 77 produced by the single very large female collected on May 16. Most of this brood died very early and the mother died also without further progeny. Excluding this one female's offspring the percentage of asexual offspring of the other 18 females is over 99 per cent. No sexual forms, either male or female, were collected, but a few ephippial eggs were secured by skimming the surface of the ponds and these on dissection seemed to be perfectly fresh, and could hardly have been unhatched eggs of the preceding season. That they were *Simocephalus* eggs is quite certain since the only other Daphnian of similar size in these ponds is *Daphnia pulex*, whose ephippia contain two eggs each.

That the practical freedom from sexual forms in this instance is due to external conditions is rendered absolutely certain by the fact that these same females when the jars were placed in the laboratory and the water no longer regularly changed, begun to produce males and sexual females, in just about the same proportions as in experiment 3 and 4, as nearly as could be judged from general observation.

Tabulated Summary of Experiment 5.

Date Collected.	Number of Mothers.	Date of Removal of Broods.	Number of Offspring.	Females.	Males.	Asexual Females.	Sexual Females.	Sex Unknown.
April 20	4	1st April 28	33	33	0	33	0	0
May 6	11	1st May 8	45	45	0	?	?	0
May 9	6	1st May 11	15	15	0	?	?	0
May 16	1	1st May 17	77	77	0	?	7	?
May 17	1	1st May 20	4	?	?	?	?	4
		2d May 23	15	15	0	15	0	0
		3d May 25	45	45	0	45	0	0
		4th May 30	51	1	50	0	1	0
		5th June 3	34	34	0	?	?	0
May 20	6	1st May 23	?	?	?	?	?	?
		2d May 26	120	118	2	?	?	0
May 23	14	1st May 25	150	150	0	149	1	0
May 25	8	1st May 30	200	198	?	?	?	2
		2d June 3	101	97	1	?	?	3
Totals......	51		890	828	53	242	9	9
Totals in 1st broods...............			524	518	0	182	8	6
Percentages of kinds of offspring in the 1st broods..................			100	98.8	0	95.6	4.3	?

V. Experiments with Ephippial Eggs.

Authors are not agreed as to the factors involved in shortening the latent period of the ephippial egg. Experimentation along this line has yielded the most inconsistent results. I have repeated the experiments of Weismann and others of freezing the eggs for varying lengths of time with all but complete failure. Hence I shall give in detail the experiments I have performed in my attempts to discover a means of ending the latent period, although in not a single instance have results been all that could be desired. In a considerable number of my experiments I have made special effort to reproduce the factors operating upon these eggs in nature, by a series of freezings, alternated with drying, placing the eggs in the sunlight, etc., but the results seem to indicate that I have neglected the one thing needful. Of one thing I am quite certain, namely, that the fact that the eggs of *Simocephalus vetulus* can be collected from the fresh-water ponds every month in the year must be taken into account in formulating any general conclusions as to the factors concerned with their development. Unless otherwise stated, eggs of *Simocephalus vetulus* were used in the following experiments. In experiments performed with eggs collected from the field there were usually several kinds present but those of *Simocephalus* predominate. My only successes have been with *Simocephalus* eggs which were produced in the laboratory.

Experiment 1.—Since the eggs that are laid in ponds are, as the ponds dry up, subjected to graduallly increasing concentrations of whatever salts may be in solution in the pond water it occurred to me that this might be a potent factor in terminating the latent period. To test this I took numerous ephippial eggs from my laboratory cultures and placed them in a pint of pond water and allowed it to stand exposed in the laboratory in a wide stender dish. I placed a graduated scale on the side of the dish, dividing the water depth into ten equal spaces. When the water had lowered to each of the various levels by evaporation, I took out a number of the eggs and placed them in covered vessels. Continuing thus until the water in the open dish had all disappeared, I left the last lot in the open vessel until it was thoroughly dry. The same experiment was tried starting with low concentrations of NaCl, but the results were negative in all cases.

Experiment 2.—A large number of ephippial eggs were collected from the surface of the ice on temporary ponds near Gary, Ind., on March 12, 1914, and dried at once. On June 20, 1916, they were placed in water at room temperature and left. They were watched for several months until it seemed certain that none would develop. On August 15 the experiment was discontinued. If freezing is the most important factor as some authors have believed, it certainly cannot be all sufficient, for this lot of eggs numbered several hundreds and they were frozen up in the ice for many weeks before they were transferred to water. At two later dates other lots were collected and treated similarly. The last was on March 21, when the ice had begun to thaw.

One lot of eggs taken from a laboratory culture in April were dried for three days, then placed in water at room temperature but did not hatch. After two months they were taken out and dried. Over two years later they were again transferred to water at room temperature without success. These experiments were performed at the University of Chicago, but before leaving there I secured great numbers of ephippial eggs by skimming the surface of the ponds near Gary, Ind., and at intervals of every few months thereafter for three years placed a number of these in water. None ever hatched. The eggs treated in this way were not counted though 1,000 would be a very conservative estimate.

Experiment 3.—This experiment was performed with ephippial eggs which had been recently produced in the laboratory cultures. They were subjected to very low concentrations of H_2SO_4, namely, M/100, M/10, M/5, M/2 and M/1, for 1, 2, 10, 24 and 72 hours. Three eggs were used for each separate set of conditions. The eggs were transferred to water and examined daily. Results were negative. A precisely similar experiment was then carried out with KOH in the place of the H_2SO_4, without success.

Experiment 4.—On January 15, 1914, 10 ephippial eggs were taken from a laboratory culture and dried for three hours, then kept in a freezing mixture for eight hours after which they were transferred to water at room temperature. None hatched.

On the same date 6 ephippial eggs were transferred directly from a laboratory culture to water which was kept at 30° C. for four weeks. Another lot was kept at 28° C. Results were negative in both cases.

On January 18, 1914, some ephippial eggs which had been produced in the latter part of December, 1913, in laboratory cultures, were subjected to the following conditions: One lot of 7 was subjected to a freezing mixture for eight hours, one lot of 6 to a freezing mixture for eight hours on two successive days, and another lot of 6 were given the same treatment on three successive days. In each case the eggs were transferred from the freezing mixture to water at room temperature. The same kind of treatment was applied to large numbers of eggs which had been collected by scraping the surface of the ice on fresh water ponds near Gary, Ind., permitting the eggs to dry between the freezings. Others were given the same treatment and then dried for three months before being transferred to water at 14° C., but all to no avail.

Experiment 5.—During the course of my work I have made numerous attempts to induce development by clipping off the small ends of the ephippia with scissors or scalpel, by pricking the shell with a needle, and by dissecting it completely off. It is hardly to be supposed that such treatment would have any effect upon eggs that had never been dried, since they often have the ventral edges of the ephippia only lightly apposed, it being possible in some instances to look between them upon the egg inside, but conceivably desiccated eggs might be thus influenced. It is quite a simple matter to spread the valves and set the egg free. I have succeeded in three instances in placing ephippial eggs with the shells removed in the brood pouch of asexual females. This did not incite development.

On August 14, 1916, I dissected the shells from 18 ephippial eggs and pricked them with an extremely fine glass needle. In some cases I barely pierced the vitelline membrane, and in others pushed the needle about one third the way through the egg. The membrane is quite tough and in most cases is under considerable tension due to osmotic pressure. There is a quantity of liquid underlying the membrane and minute quantities of this can be seen to exude from the wound in all cases, and if the wound is made with too large a needle or too deeply the granular cytoplasm is extruded. Presumably such eggs are destroyed. Fourteen of these eggs seemed to have been successfully treated and were placed in a stender dish with enough algæ to nourish any embryos that might appear. They were examined daily for some weeks and at intervals for two months. Some of the eggs degenerated early and others persisted as if no injury had been received. None had developed on October 10, and shortly thereafter no eggs could be found.

Experiment 6.—On January 15, 1916, two dozen eggs were taken from a laboratory culture which had been producing an abundance of them, and without being allowed to dry, were placed in a vessel where the sun would strike them for a few hours daily, for some weeks. They were then placed out of the sun. On August 16 I placed them on ice for 24 hours, and then left them until June, 1917. That they were in perfect condition at this time was shown by the fresh green color of the eggs on removal of the ephippia. They were held under water by being placed in a vial which was left lying on its side in a stender dish. None of these developed.

Experiment 7.—On June 30, 1916, a number of ephippial eggs of *Daphnia pulex* were taken from a laboratory culture and placed under water in the same manner as in the above experiment and left thus submerged until May 20, 1917. Dissection of the ephippia from several of these showed them to be in good condition at the end of this time, none having developed.

Experiment 8.—Although all of my carefully devised attempts to induce ephippial eggs to hatch by the application of chemical and mechanical stimuli, as well as the numerous repetitions of the freezing experiments of Weismann, have completely failed, I have succeeded in securing about 70 stem mothers from some *Simocephalus* vetulus eggs which had been given no special treatment. On June 27, 1916, I removed several hundred eggs from a culture which had produced great numbers of them and placed them in a large-mouthed 8 oz. bottle lying in a horizontal position in a one-gallon battery jar about one third full of water. A small amount of unicellular green algæ was added. Cypris of several species soon appeared in large numbers. On August 14 an embryo of *Simocephalus* appeared. I removed it in a little of the same water together with some of the algæ. It lived only three days. Fortunately others were to follow. On August 18 another appeared, and produced a brood of 7 females on August 24. Eight more hatched on August 21, 7 more on August 25, 10 on August 26, 6 on August 30, 4 on September 3, 2 September 6, 1 September 20, and at more or less irregular intervals for several months thereafter others continued to appear, until nearly six dozen had been secured.

Two other instances deserve mention. Three days before setting up this experiment I had placed a number of eggs, produced from May 1, to June 26, in a jar in a similar manner and they remained there until December 12, when they suddenly began to hatch, but only a few appeared. I can suggest no explanation as to why they did not all hatch, or why the few that did should not have done so earlier as did most of those mentioned above. On August 9 some fresh eggs were placed on ice and left 36 hours and then placed under water in the manner described above. On December 27 two hatched. No more appeared although the experiment was left standing until June 1, 1917, *i.e.*, about 11 months.

Mortality among these stem mothers was found to be about the same as with other females. I succeeded in obtaining an average of a little over six broods from each of 24 of these stem mothers and have given their history in the interesting experiment (number 3) already described.

It should be stated that there were numerous other eggs in the culture from which the majority of the stem mothers was secured, which did not hatch. Some of the water from this culture was removed and other newly produced eggs placed in it. This experiment was left standing from August 30, 1916, until May 5, 1917. Since none of these developed we must infer that the kind of water in the battery jar containing the developing eggs, was not the responsible factor, though I have not the remotest idea what it may have been.

VI. Observations on Pairing.

A female from a culture containing many ephippial females was isolated on July 19. By July 27 she had produced two female broods, one of 14 and another of 10. I placed the older brood with males. On August 1st I found that 14 ephippia had been produced, though 10 of them were empty, and all but one of the females had become asexual. The one sexual female remained so until she had developed three ephippia. Then she developed a brood of 5 asexual females and died August 9. It was not surprising to find so large a percentage of empty ephippia appearing in the presence of males since the same thing occurs in general cultures, though I had thought it might, in the latter case, be due to too small a percentage of males. That this explanation is erroneous is shown by this and the following experiments.

Several of the female broods obtained from the two females isolated in isolation experiment number 1, were placed with numerous males and examined daily. On July 1 more males were added since I noticed several incipient ephippia appearing. On July 30 the culture numbered about 150 individuals, and only two ephippia had fully developed, one of these being empty. By August 6 there had been developed 14 ephippia, most of them empty. The percentage of ephippial females thus remained less than 10 per cent., so low that it is quite obvious that the presence of males could not be the factor responsible for their appearance, since larger proportions of sexual females often occur in females which are segregated from the males. Not all of the ephippia reached full size, though most of them did. There was a dearth of males at no time during this experiment, yet less than half of the fully developed ephippia contained fertilized eggs. The fourth brood of female number 18 of isolation experiment 3, consisted of 7 females. These when very young were placed with 10 males. All seven, however, proved to be asexual, although some of their offspring developed into sexual females in the absence of males.

The 20 females of the fifth brood of female number 38, of isolation experiment 2, were divided into two lots, 10 being kept with males and 10 without. Of the 10 with males, 9 developed ephippia, a few of which contained eggs when the molt was cast. These eggs, however all degenerated, so presumably they had not been fertilized. The males had all died at this time. Six of these females became asexual after producing the first ephippium. I placed some more males with the remaining four sexual females and was fortunate enough to observe two matings of the same female within two hours. I saw no spermatozoa lost in these instances as I have in several cases observed later, and I have no doubt that the one normal ephippial egg which I found later was the result of this pairing.

Of the 10 without males, one died early, 5 developed ephippia, and 4 became asexual at once. The 5 sexual females developed in all 16 ephippia, which is more, and more per individual, than was produced by those kept with males. Males appeared in some of the broods of the asexual females, but most of the ephippia were produced before they appeared, and there was only one fertilized egg. The sexual females all became asexual before death.

The 35 females of the sixth brood of this same female number 38, were also saved. The one male of the brood died at the age of four days. Of the 16 ephippia produced by the members of this brood one contained an egg. Since the egg did not degenerate it must have been fertilized. Thus we have proof of the early functioning of the males. They are shorter lived than the females, or at least that has been the case with individuals isolated. It will be noted that this brood contained three kinds of individuals, namely asexual females, sexual females, and a male. My records contain numerous instances of this kind.

All of the females of broods 4, 5, 8 and 11 of female number 41 of isolation experiment 3, were placed with males and a complete record kept of the results. The fourth brood consisted of 7 males and 7 females, all sexual. In all 17 ephippia were cast by these 7 females. Two of them then died and the remainder became asexual. In spite of the presence of the males 15 out of these 17 ephippia contained no egg. The fifth brood consisted of 5 males and two sexual females. These were reared together. Each female produced a fertilized egg and one of them an empty ephippium afterward, this one then dying. The other became asexual though the males were continually present. The eighth brood consisted of 20 females. A number of males were placed with these. Five of the females died early, and none of the rest developed even an incipient ephippium. The eleventh brood consisted of 3 females, 2 being sexual and 1 asexual. The asexual one was discarded. These two females whose mother was asexual were reared under conditions which were as nearly alike as they could possibly be made and yet have them in two separate vessels, gave rise to diverse offspring. An equal number of males was placed with each. The history of one was: an empty ephippium, 4 males, 4 sexual and 2 asexual females, an undetermined brood, death. The history of the other was: an empty ephippium, a fertilized egg, a fertilized egg, 3 sexual and 3 asexual females, 1 sexual and 4 asexual females, death. Each produces an empty ephippium at first, but the former then produces a brood of 4 males, and the latter another ephippium, this time containing a fertilized egg. Both later produce both kinds of females. Several other experiments of this same kind gave similar results. I wish to point out here only one conclusion based on these experiments, namely, that all afford evidence that the reason for the production of male broods is not the production of ephippial eggs which were not fertilized because of the absence of males, for in these experiments the onset of male brood production has been observed many times in the females which have passed from the sexual state and begun the production of males, in the presence of other males.

The method of copulation is interesting. This was observed in several instances. The sixth brood of female number 44, of isolation experiment 2, consisted of 8 sexual females and 1 asexual female. When the first ephippia of these 8 sexual females were in their incipiency I introduced 5 males and watched them almost continuously, removing them when I could not give them attention. When the females were about 6 days old the ephippia were well developed and the ephippial eggs seemed to be ready to be extruded into the brood pouch. It was at this time that the monotony of proceeding was relieved. On being placed in the vessel with the females the males at once became intensely excited. This lasted nearly an hour. After several abortive attempts at pairing one pair mated. The male opened his carapace slightly and clasped the antero-ventral margin of one side of that of the female. He then bent his abdomen ventrad, so that it extended between the ventral margins of the female's carapace, ventral to her posterior appendages. In this position he waved his abdomen about for a few seconds, then straightening it, he ejected the spermatozoa in two forcible streams, which, like puffs of smoke, spread abruptly when their force was spent. They seemed to me to be all swept away by the exhalant respiratory current of the female. The excitement of the males was now abated, and in about fifteen minutes I removed the males. Two hours later I introduced the males again and secured a second mating. In this instance the male ejected the spermatozoa just anterior to the posterior end of the abdomen of the female, and posterior to her last pair of appendages. Again I saw some of the sperm cells swept away, but very few. I am now convinced that the first case described was abortive. Other observations were made later and in some cases no part of the mass of spermatozoa was lost, though no case was observed in which the sperm cells were not ejected freely into the respiratory chamber as described, the only point of contact being the ventral margins of the male's carapace with the antero-ventral margin of one side of that of the female. The ephippial egg is laid within about an hour, and in no case observed more than two hours, after the pairing. During the period of œstruation the female doubtless produces some chemical substance which passes out in her exhalent respiratory current, since it is by passing through this that the males are made aware of the females' readiness to mate.

I have made oft repeated attempts to observe the copulation process from the lateral aspect. Several devices, such as a trough made of cover glasses and a slide, in which the specimens were placed, yielded only failures. The males do not always attach to the same side of the female's carapace. In case the male attaches himself to the left side of the female's carapace, the spermatozoa are ejected near that side of the respiratory chamber and pass to the left of her abdomen to the brood pouch, seemingly being driven dorsad by the action of her last pair of appendages. In one such case observed the ephippial egg was developed in the right ovary. In order to enter the oviduct the spermatozoa would have to pass across the brood pouch to the right side. The sperm cells are immotile. Though I have not yet been able to see them after they have been transferred to the brood pouch, I am convinced that they are retained in it and that fertilization takes place after the egg is laid.

VII. General Discussion.

1. What relation does the presence of the males bear to the origin and prolongation of the duration of the sexual state in the females?

One gets the impression from the literature that the ephippial egg and ephippium are completely developed only if fecundation occurs. Perhaps enough data have already been given in the above discussion of the observations on pairing, of the effect of the presence of males in prolonging the duration of the sexual state of the females to convince any one that it is nil, though more evidence is at hand. I wish to add here that I have observed some hundreds of instances of embryo females developing through the stage at which they would become sexual, if at all, which never evinced the slightest evidence of sexuality. In a great many instances these were in the same containers with sister females which attained a high degree of sexuality. The males used in some of these experiments were related to the females, but in most instances they were not. It is evident that the ephippial egg and the ephippium are both developed to within one or two hours of the time of extrusion of the egg into the brood pouch, in complete independence of the male, the only definite correlations of these processes with the presence of males to be noted, being that the egg is usually not laid in the absence of fecundation, the exceptions being, as I should judge, about 1 per cent. to 5 per cent. I have observed about half a dozen instances.

2. In the same environment are individuals which pass through the sexual phase any more likely to give rise to males and sexual females than are individuals which are parthenogenetic from the beginning of their reproductive period?

Since the twenty-fourth female in isolation experiment 3 gave rise to pure broods of males, pure broods of sexual females, pure broods of asexual females, to mixed broods of males and females, and to pure broods of females of diverse sexuality, thus compassing the whole range of possibility as to kinds of offspring, she was presumably normal, hence I used some of her sexual offspring with which to test this question. I selected 12 of the sexual females of broods 12 and 13. Each of these 12, after having produced one empty ephippium at the age of 11 days, became asexual. Two of the 12 died. In two other cases the first brood died too early for identification, but the first determinable broods of the 10 that lived were as follows:

1. 6 females, 1 sexual, 3 asexual, and 2 undetermined.
2. 7 " 2 " 3 " " 2 "
3. 3 " 0 " 2 " " 2 "
4. 3 " 0 " 3 "
5. 2 " 0 " 2 "
6. 10 " 4 " 6 "
7. 6 " 1 " 5 "
8. 3 " 1 " 2 "
9. 12 " 1 " 11 "
10. 6 males

Thus in these first broods of the 10 sexual mothers only about 22 per cent. of the females are sexual, and about 12 per cent. of the offspring is male. All of the subsequent broods of these 10 mothers were also saved and identified. It was found that the percentage of males was slightly over 8 per cent., a decrease from that shown in the broods immediately following the ephippia. This can be of no special significance however since the percentage in that case is very much lower than one usually finds even in the offspring of asexual mothers, as noted in other experiments. As we have already seen, somewhat over 40 per cent. of the offspring, totaling over 1,700, of the 45 females concerned in isolation experiment number 2, is male. About 33 per cent. of the broods of these 10 sexual mothers are mixed as to sexuality, which is about normal. It will be recalled also that the percentage of males and sexual females in the sexual line of offspring from the stem mothers, (see isolation experiment 3) is actually slightly less than in the asexual line. It thus seems certain that the production of ephippial eggs at the beginning of the reproductive period, whether they are fertilized or not, has no influence on the subsequent offspring.

3. Is the age of the mother correlated in any way with the kind of offspring?

The twenty-fourth female in isolation experiment 2, gave as her first two broods, pure male offspring. These were followed by three pure broods of females, and then a brood of 15 males. The seventh brood was a mixed brood. Her last 7 broods, totaling 66, were all females. The sexual and asexual females in these last broods occured in about equal number, namely, 30 sexual, and 36 asexual. Numberless such instances might be cited to show that males often appear in the first broods. The summary of the data in isolation experiment 2 shows that the first broods were pure male in 16 instances, pure female in 26 instances, mixed in 2 instances, and undetermined in 1; while the last broods were pure male in 11 instances, pure female in 28, and mixed in 6. The tabulated results in isolation experiment 3 are also in accord with the data just given and are, I believe, quite conclusive on this point. It will be noted that there is no marked increase or decrease in the number of males, sexual females, or of asexual females, from the first of the sixth broods, inclusive, in the descendants of the 24 stem mothers, for the three generations, in either the sexual or asexual lines. When the first broods of newly collected old and young females are compared no difference in kind is noted, as shown in experiment 5. When these same individuals are kept in small containers in the laboratory, no matter if the food supply is abundant, if the water is not changed often, sexual forms appear in the offspring of young and old alike, and in like proportions. These facts point to the conclusion that there is no correlation between the age of the mother and the kind of offspring.

4. Do mixed broods indicate a transitional stage from male producing to female producing and vice versa?

The summary of isolation experiment 2 shows 9 instances of the production of mixed broods which are preceded and followed by broods of the same sex, while there are but four cases in which mixed broods are preceded and followed by broods of different sex. There are in this same experiment two cases in which the first brood was a mixed brood, and 6 cases in which the last brood was mixed. Quite often one mixed brood follows another. I have found no evidence in favor of the view suggested by this question, the occurrence of mixed broods in these experiments being entirely capricious.

5. What relation has sexuality to the duration and senescence of the laboratory cultures?

The fact that my cultures which have produced sexual females and males have always passed sooner or later into a more or less non-productive phase and did not die out provided that conditions were not so severe as to terminate them, is well deserving of consideration, in view of the fact that one so often reads in Daphnian literature of cultures becoming sexual and being thereby terminated. During the five years I have worked on *Simocephalus* I have run scores of cultures and I have never had an instance of a pure sexual culture except those set up by selecting males and sexual females from other cultures. At the most such cultures remain purely sexual only a few days, when with the passing of some of the sexual females into the asexual phase, broods invariably appear containing asexual females. I have no record of a large pure brood of sexual females, and no record of a female which has consistently produced pure sexual small broods. Broods containing sexual females may be produced by any female no matter what her pedigree has been. Naturally if a large percentage of the females in a culture are passing through the sexual phase, so much of their immediate productivity is sacrificed, but since the number of ephippial eggs produced rarely exceeds 4, and is usually only 1 or 2, and always being produced within the first 10 to 25 days of their lives, every sexual female devotes the most of her life to asexual reproduction. Old cultures, in which there has been much accumulation of excreta, run down, the size of the broods decreases to from 1 to 5, many individuals die before maturity, and the culture dies out finally; but so far as I am able to see, this neither causes nor results from the production of ephippial eggs.

It is of interest to consider what happens in laboratory cultures which are set up and allowed to run their natural course unhindered. With each female producing a brood of from 3 to 35 every 40 or 50 hours, as is always the case in a culture containing an abundance of fresh green algæ and no accumulation of excreta, any culture, even though started with a single individual, will become overstocked in a few weeks, and the increase in numbers has to stop, the development of eggs in the ovaries and the growth of the embryos become retarded, the culture remains nearly at a standstill for some weeks, and if the food supply is exhausted will finally die out, though it has been my experience that they usually linger on indefinitely. By the time the culture has become overstocked ephippial eggs have appeared in considerable numbers, and after having passed the state of equilibrium, when the rate of reproduction has rather abruptly ceased, for some weeks just about but not quite equaling the death rate, the accumulation of ephippial eggs may be taken as proof that the culture has passed through a sexual phase, which in turn is likely to be erroneously considered the cause of the decline of the culture. And now with reproduction at its lowest ebb, and all of the sexual females having passed into the asexual state, as they all do, the observer is naturally likely to believe that the culture has passed into the asexual phase, since all of the sexual members of the culture have yet to live the asexual phase of their lives. Thus there will be an indefinite period when nearly every member in the culture will be asexual. It must be remembered that at this critical period, assuming the ordinary proportion of males, reproduction must be cut down to somewhat less than two during the life time, which is a few weeks or months, of each female. Considering that 25 per cent. of the females are sexual (see table, isolation experiment 3), and that each sexual female is actually carrying an ephippium one-fifth of her life time, even assuming that to be but 14 days it is obvious that only 5 per cent. of the females will be carrying ephippia at any given time. I believe the life time in a large culture is in reality much longer than I have assumed, and 25 per cent. far too great, and that it is safe to say that in many instances the ratio of females actually carrying ephippia at a given time is as low as 2 per cent. If it should happen that these few should cast their ephippia at the same time, for a day or so thereafter one could find a sexual individual only by a tedious hunt with the microscope.

On the other hand, when reproduction proceeds apace, starting with 10 females let us say, each producing only 6 broods, with an average of 1 and $\frac{3}{10}$ sexual females to a brood, (as was the case in isolation experiment 3), we should have 78 ephippial females in the first generation (10 × 6 × 1.3 = 78); and supposing there were in all only 5 females to a brood, (see table of same experiment), we should have 300 females to mother the F_2 generation, which should yield 23,400 ephippial females, out of a total of 90,000. Of course in practice, laboratory cultures are overstocked long before all of the second generation has appeared, and in fact reproduction has practically ceased by the time 100 to 200 ephippia have appeared. The accumulation of the ephippia makes the culture seem to be in the sexual phase. The variation in the number of kinds of offspring produced may depend to some extent upon the extreme prolificacy and consequent mortality in laboratory cultures, since the assumption of ever so slight a difference in the susceptibility of embryos to the great variety of adverse conditions met with in laboratory cultures, would be sufficient to account for much of the seeming variation. One may sometimes find several hundred ephippia in a laboratory culture in which there are very few males. In one such instance I collected 200 ephippia, only 2 of them containing eggs. Four explanations are possible: they may have hatched, which is very unlikely, there may have been too few males to fertilize them, mating may have been inhibited by some unfavorable cultural condition, or, there may have been abnormal pairing such as I have described in the section on pairing, in which case the spermatozoa not being retained, the eggs would degenerate. I have had a few small cultures in which for a short time males were very numerous and sexual females almost absent, but such instances are rare.

The following is typical of my experience with *Simocephalus*. I isolated a female on July 1, 1913, and kept her in lake water from lake Michigan. This was changed daily. She had nothing to eat except what she could get from the lake water. This seemed barely sufficient since reproduction proceeded very slowly. On August 1 I placed 6 of her descendants in a rich algæ culture. Here they multiplied very rapidly. By August 13 there were 50 ephippial eggs floating on the surface, and males were also present in the offspring. This culture continued to produce asexual forms for some months, when the algæ gradually died out individuals in the culture became less numerous, reproduction having practically ceased. Another culture was established from this by selecting a few specimens at random and giving them a fresh supply of algæ. It ran through a similar period of great productivity, about the same number of sexual forms occurring, and declined as in the first instance. A third fresh culture was stocked by a single specimen from the last. It likewise gave rise to a great profusion of Daphnians, sexual forms appearing in considerable numbers. Unfortunately I did not keep a record of the time it took in the last two instances for the sexual forms to appear; but, as shown above, the first culture, started August 1, produced 50 ephippial eggs by August 13, and that in the presence of an enormous quantity of green algæ, the container being a three gallon jar which had previously been used for *Paramecia*. This *Paramecium* culture had been started with boiled wheat as the source of nutrition and had been permitted to stand for nearly a year. The *Paramecia* had disappeared and green algæ had developed in immense quantities on the side of the jar away from the light. Dearth of food could not have been a factor in inducing sexuality in this culture. I have already shown in isolation experiment 2 that sexuality comes on in spite of an abundance of food, in small vessels containing only a single individual female. The study of general cultures leads to conclusions which are in agreement with results obtained by a study of isolated females, namely, that onset of sexuality is independent of food shortage, and suggests that it is related to accumulations of certain excretions, which become critical in their effect upon the kind of offspring surprisingly early in *Simocephalus* cultures which are allowed to run their natural course unhindered. Production of ephippial eggs reaches its highest level about the time a general culture is over stocked and the rate of reproduction has begun to decrease, passing to lower levels as the food supply decreases, as is readily demonstrable if one will but take the trouble to remove them as they appear in such a culture, recording the daily output.

Of all the females isolated for study and kept in small containers in the laboratory, including several hundred selected at random and as many more whose pedigree was known for several generations, also 60 stem mothers and all of their female offspring that lived, for three generations, the only instances in which both males and females did not appear in some of the broods were those in which they were very few, *i.e.*, in which the isolated females died early. The same was true of sexual females. They always appeared in some of the broods unless they were too few. Pure broods of sexual females were very rare and they have never predominated in any of my cultures no matter how severe the conditions were. Though I have not carried out a definite experiment to determine how many generations a culture may be carried through the sexual females alone (except in isolation experiment 3, where distinct lines were carried to the third generation) I have not the slightest reason to suppose that they may not be carried on indefinitely, since each female, whether sexual or asexual, gives rise to both sexual and asexual females, provided only that the food supply and other cultural conditions have proper attention. I am convinced that much of the confusion in the literature on the relation of sexuality to the senescence of cultures is due simply to the extreme prolificacy of the Daphnians and the complex phenomena resulting therefrom. Sexual phase and asexual phase may well be applied to individuals, and asexual phase to cultures, but in *Simocephalus vetulus* cultures never become wholly sexual, only partially so, since in the most sexual of cultures the offspring of a given female are always mostly asexual, and the sexual females pass most of their lives in the asexual state. Finally let us recall that at the extremely modest rate of production of ephippial eggs assumed in our calculations above, that starting with 10 females we should have 23,400 ephippia at the end of the second generation, whereas, as a matter of fact the most sexual of cultures of *Simocephalus vetulus* produces only a few during a course of many months. Hence it is folly to consider the onset of sexuality causally related to senescence of cultures in this species.

6. Does *Simocephalus vetulus* depend upon external factors to call into expression maleness, sexuality in the females, and parthenogenesis?

With respect to this point the results of Grosvenor and Smith (1913), Banta (1914), and Agar (1914b) are particularly instructive. They succeeded in completely inhibiting sexual forms in several genera of Cladocerans for an indefinite number of generations. Their conclusions are more fully discussed under the head of literature review.

In my earlier experiments there are numerous instances of the production of all three kinds of individuals, namely, males, sexual females and asexual females, by the same mother. Less common is the production of only two kinds of offspring during the life time of a female. In these experiments I have no record of a female producing only one kind of offspring except those whose progeny are very limited because of the early death of the mother, and in recent experiments, those which produce only asexual females, this last being of much importance. Rarest of all yet quite often met with is the production of all three kinds of offspring in the same brood. Female number 45 of isolation experiment 2 has one such brood containing only three embryos. Broods of this type are usually larger. The asexual females have nearly always outnumbered the sexual females and males in mixed broods, as they usually do in the sum of the broods of a given female.

Similar proportions of the kinds of offspring may be secured under a great variety of kinds of environment. In the most highly sexual cultures many pure asexual females are always present, and the sexual females remain so only for a brief period in early life. While these facts do not prove that the environment has nothing to do with the ratio of kinds of offspring, they would seem to indicate that it is not the determinative factor in the nexus of causes that are responsible for the expression of the species in all of its forms, regardless of internal factors.

VIII. Summary.

I. *Life History.*

1. The offspring of any female of *Simocephalus vetulus* in the asexual phase may consist of any one or all three of the following kinds of individuals:

(*a*) Sexual females, which produce a series of from one to seldom more than six ephippial eggs early in life, then become parthenogenetic and so remain, being then indistinguishable from other parthenogenetic females. The final ephippium is often only partially developed, showing the sexual capacity to be gradually lost.

(*b*) Parthenogenetic females, which display no tendency to produce ephippial eggs.

(*c*) Males.

The kinds of offspring occur in no definite order, but their character is probably determined at birth, and not by subsequent conditions. (See summaries of isolation experiments 2 and 3.) Eggs which will develop into males, into highly sexually females, and parthenogenetic females, often arise in the same female and not infrequently at the same time, either early or late in her reproductive period, whether or not she has passed through the sexual state herself.

2. The sequence of the generations is very indefinite:

(*a*) The stem mother is functionally like the females produced parthenogenetically, except that she probably never gives rise to ephippial eggs. (However see Sharfenberg, 1911, p. 24.) There is not even an approximately definite number of generations in the cycle from one stem mother to another. It may be one or many.

(*b*) The remoteness of the generations from the stem mother bears no definite relation to the percentage of males produced, the ratio of sexual to parthenogenetic females, or to the duration of the sexual state when present.

(*c*) The sexual state is probably determined in the ovary of the preceding generation. There are almost certainly predisposing factors in the environment but it is not certainly known what they are. Food or lack of food does not offer a sufficient explanation. Sexual females and males tend to arise at the same time, presumably in response to the same environmental complex.

(*d*) Cultures are indefinitely viable parthenogenetically. The species will express itself in all of its forms under a great variety of conditions. Under certain conditions sexual forms are completely inhibited. Parthenogenesis cannot be completely inhibited in cultures or even in individual females. Thus cultures of *Simocephalus vetulus* can never be terminated merely because of the onset of sexuality.

(*e*) The production of mixed broods is not to be interpreted as evidence that the female producing them is undergoing transition from male producing to female producing or vice versa.

3. The production of ephippia and ephippial eggs are related but not causally, both being dependent upon common internal factors. The introduction of males into a culture does not induce the production of ephippial eggs, nor does their presence have any relation to the prolongation of the sexual state when it has once appeared.

II. *Breeding Habits.*

Sexual attraction is limited not only to sexual females in the sexual state, but is confined to a limited period of a few hours before the ephippial egg is laid. It seems to be due to some kind of substance omitted by the female and borne by her exhalant respiratory current, where it is detected through a chemical sense by the male. Fertilization seems to take place in the brood pouch after the egg is laid. The presence of the spermatozoa in the brood chamber is probably the stimulus for its extrusion. In the absence of fertilization the ephippial eggs are usually resorbed in the ovary, or, if laid they undergo degeneration in the ephippium within one or two days.

III. *Theoretical.*

The immediate significance of the ephippial egg is as a stage in the life cycle resistant to adverse conditions, and not in the stem mother hatching from it, since her offspring are in no way unique. A more remote but more fundamental significance, held in common of course, with all fertilized eggs and zygotes, is that it provides for the permanent lability of the species through amphimixis. In view of the great prolificacy of the species in regard to all of its forms, and the almost universally concomitant occurrence of males and sexual females, the development of only 1 per cent. of the ephippial eggs would be quite sufficient to secure to the species all of the benefits to be derived from their two functions. It seems probable that there is a very great inherent variability in the capacity for development of the ephippial eggs in a state of nature, and the lack of uniformity of results obtained by the various investigators in their attempts to shorten the latent period may be explainable on that basis.

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MITOTIC DIVISION OF BINUCLEATE CELLS.

ETHEL BROWNE HARVEY.

Attention has been called to the presence of binucleate cells in the follicular epithelium of insects by several observers (Korschelt, '87, Preusse, '95, De Bruyne, '97, Gross, 01, et al.). In *Notonecta*, binucleate cells are characteristic of the follicular epithelium and also of the tissues lining the sperm duct and investing the spermatogonial cysts. These binucleate cells undergo normal mitotic division, both nuclei being involved. As a usual rule, both nuclei pass through the stages of division synchronously, though rarely one finds one nucleus in advance of the other. (Fig. 11, upper cell.)

In the resting stage (Fig. 1), each nucleus is exactly similar to the single nucleus of a spermatogonial or oögonial cell, a conspicuous karyosphere being present (Browne, '13). In the prophase, the two nuclei remain distinct and thick chromatin strands are present in each nucleus (Fig. 2, upper cell, Fig. 3). When the nuclear wall breaks down in the late prophase (Fig. 4), the two nuclei may undergo a certain amount of fusion, or they may remain entirely distinct and this is true also of the metaphase. In some cases, two distinct plates may be seen in polar view (Fig. 5) or two distinct spindles in side view (Fig. 6); in fact, the two spindles may be so independent as to lie in different planes, so that one is seen in side view and the other in polar view (Fig, 7). In other cases, the two spindles become so intimately combined as to appear as one giant spindle. In polar view a perfect equatorial plate is seen, but there are *twice as many chromosomes present as the diploid number for the species.* Each chromosome of the spermatogonial or oögonial metaphase is represented by two exactly similar chromosomes in these binuclear plates, as may be seen by comparing Fig. 8 from an oögonial cell of *Notonecta indica*, with Fig. 2 (lower cell) from a follicle cell of the same species. In the oögonial cell, there are 6 large, 4 small and 16 intermediate sized chromosomes, in the binucleate follicle cell there are 12 large, 8 small and 32 intermediate ones. Similar double chromosome plates have been found in the follicle cells of other insects, *e.g.*, *Anasa* (Wilson, '06) and are probably likewise due to the division of a binucleate cell.

In the anaphase (Fig. 9), the two sets of chromosomes cannot be distinguished, and the two spindles appear always to be fused. It is difficult to tell exactly what happens in the telophase. It would seem that a membrane formed around the group of chromosomes at each pole, (Fig. 10), each daughter nucleus becoming elongated and that it later separated off into two nuclei (Figs. 11 and 12). It seems entirely possible that in such a separation into two nuclei, which would resemble a nuclear amitosis, the chromosome content might be so distributed that each nucleus would maintain the chromosome constitution characteristic of the species. It is possible that the separation into two nuclei occurs only in those cases where the original sister nuclei did not become entirely fused and that when they are fused, each daughter nucleus remains as an abnormally large nucleus, really double in nature. It is evident, however, from the series of figures that two binucleate cells may arise by the mitotic division of a binucleate cell.

Although the binucleate cells of the follicular epithelium have been believed by some observers (Preusse, '95, De Bruyne, '97, Gross, '01), to arise by an amitotic division, I have found no evidence of such an origin, and would believe it probable that they arise by the failure of cell division after a nuclear division by mitosis. Macklin ('16) has described a process of amitotic nuclear division in his study of binucleate cells in living tissue, but the figures he gives might also be interpreted as the last stages in the mitotic division of a binucleate cell as described above. If binucleate cells do arise as Macklin believes and his interpretation of them as potentially mononuclear, is correct, there must be two types of binucleate cells. In one type, each of the two nuclei has a complete set of chromosomes, as described above. In the other type, the two nuclei taken together have a complete set of chromosomes. Macklin was, however, unfortunately unable to verify his assumption as to the chromosome constitution of his binucleate cells. Binucleate cells, arising by the suppression of cell division after nuclear division by mitosis have also been produced experimentally in the cleavage cells of *Crepidula* (Conklin, '12). The two nuclei may divide mitotically and entirely independently although synchronously; in these cases, however, two mononucleate cells are usually produced from the original binucleate cell.

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EXPLANATION OF PLATE I.

Fig. 1 from the sperm duct of *Notonecta shooterii;* Fig. 8 from oögonial cell of *N. indica*, other figures from follicle cells of *N. indica.*

FIG. 1. Binucleate cells from sperm duct of *N. shooterii*, resting stage.

FIG. 2. Upper cell, prophase. Lower cell, metaphase showing a perfect double chromosome group, 52 chromosomes.

FIG. 3. Prophase.

FIG. 4. Late prophase.

FIG. 5. Metaphase, polar view, two plates separate.

FIG. 6. Metaphase, side view, two plates separate.

FIG. 7. Metaphase, two spindles in different planes.

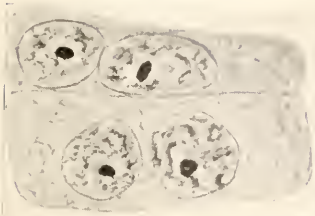
FIG. 8. Metaphase of oögonial cell of *N. indica*, showing 26 chromosomes.

FIG. 9. Anaphase.

FIG. 10. Telophase.

FIG. 11. Lower cell, telophase, sister plates dividing into two. Upper cell one nucleus in prophase, one in metaphase.

FIG. 12. Late telophase showing two daughter binucleate cells.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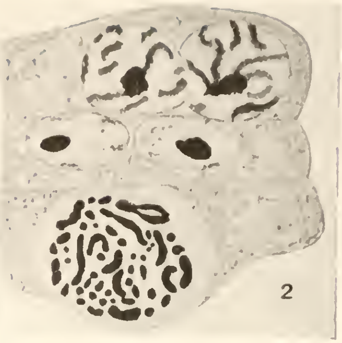
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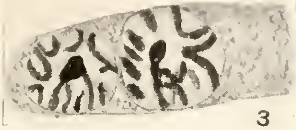
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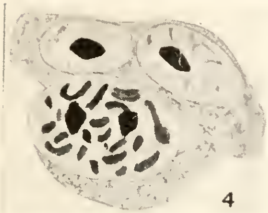
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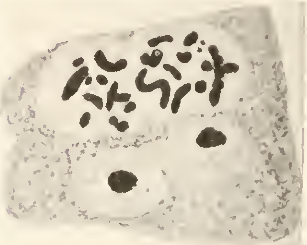
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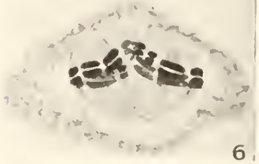
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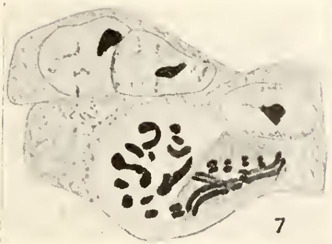
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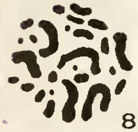
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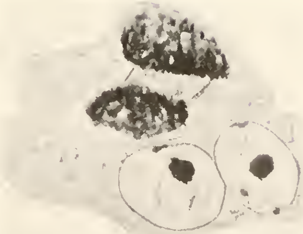
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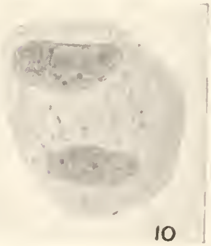
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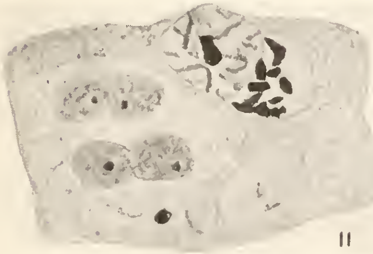
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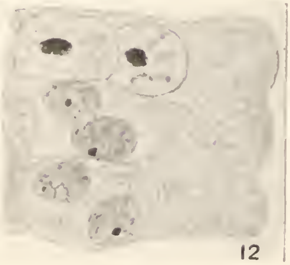
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ETHEL BROWNE HARVEY.

THE AXIAL GRADIENTS IN HYDROZOA.

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II. SUSCEPTIBILITY IN RELATION TO PHYSIOLOGICAL AXES, REGIONS OF COLONY AND STAGES OF DEVELOPMENT IN CERTAIN HYDROIDS.

The present paper is a study of the susceptibility relations in certain species of colonial hydroids and includes data bearing upon the following points: axial susceptibility in single hydranths, medusa buds and stems; susceptibility in relation to stage of development, physiological age and motility; regional differences in susceptibility in colonies and their relation to growth form and general physiological condition; susceptibility in acclimation. These data have been accumulated during some five years past, in part at Woods Hole and in part at Friday Harbor, Wash. The forms chiefly studied are *Pennaria tiarella* and *Tubularia crocea* at Woods Hole and *Bougainvillea* sp., *Obelia geniculata* and *O. borealis* at Friday Harbor. Some work was also done with a third unidentified species of *Obelia* and with another campanularian, *Gonothyria clarkii*, also at Friday Harbor. I am indebted to Professor Trevor Kincaid and to Professor C. C. Nutting for identification of certain of the Friday Harbor species.

For the direct determination of susceptibility to slowly lethal concentrations of agents the method is the same as that used for *Hydra* (Child and Hyman, '19) and many other forms, viz., the determination of the time of death as indicated by the disintegration or cytolysis of the protoplasm in KNC, HCl, various anesthetics, neutral red, methylene blue, or in some cases by the time before decoloration in KNC or other neutral or alkaline agents after staining with neutral red. This decoloration occurs only when the protoplasmic structure disintegrates: the first stage is a change in color of the neutral red to yellow as the sea water or the alkaline agent penetrates all parts of the protoplasm, and following this the yellow color gradually disappears in most cases, the dye being no longer held by the dead protoplasm. The vital dyes, neutral red and methylene blue have been used both as agents for staining intra vitam and for determining differences in susceptibility. In the investigation of acclimation in lower concentrations of KNC, HCl, ethyl urethane, $MgSO_4$, neutral red and methylene blue, it was found that different hydranths or regions of the colony show differences in capacity for acclimation.

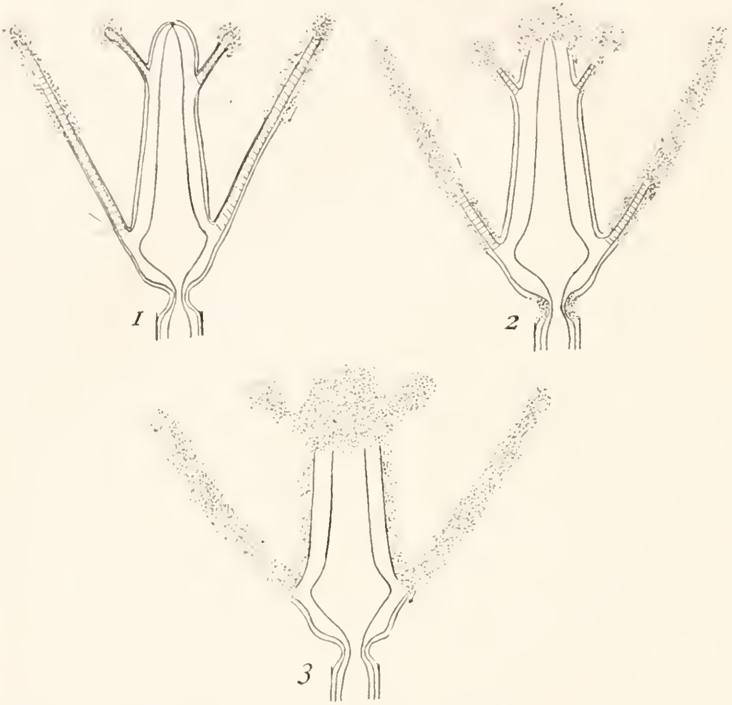
The question of the value of susceptibility as a criterion of certain quantitative aspects of physiological condition has been discussed so many times in earlier papers (see, for example, Child, '19*b*, and the references there given), that no further consideration of the matter is necessary here. It need only be said that a rapidly increasing volume of evidence along many different lines indicates that in general susceptibility to higher concentrations or intensities of at least many agents, as indicated by death or inhibition, as well as capacity for acclimation to, or recovery from the action of lower concentrations or intensities, vary in general, though not necessarily proportionally to the rate at which fundamental physico-chemical processes are going on in protoplasm.

Since adequate presentation of the experimental protocols on susceptibility would require much space, only the general results are stated, but for each statement made there is an extensive experimental basis. Moreover, the experiments demand no difficult technique and can easily be repeated, the chief precaution to be observed being the distinction between freshly collected material in good condition and that which has been kept in the laboratory. With some species even a few hours under laboratory conditions are sufficient to alter the susceptibility relations of different parts to a considerable degree.

The Death Gradients in Hydranths.

The observations on hydranths have been made repeatedly, both on the different hydranths of single colonies and different colonies of the species mentioned above, and the progress of disintegration has been observed in hundreds of cases. The hydranth of *Pennaria tiarella* will serve as an example of the course of disintegration in the hydranth of a tubularian hydroid. In full grown hydranths in KNC *m*/800 to *m*/400 approximately, in neutral red, or in KNC after staining with neutral red to make the death changes more clearly visible, disintegration begins in the ectoderm at the tips of the proximal tentacles and progresses basipetally, usually at about the same rate in each tentacle (Fig. 1). Some time later, usually when disintegration has progressed over one to two thirds the length of the proximal tentacles, it begins in the ectoderm at the tips of the distal tentacles and progresses basipetally in these also, reaching the bases of both series of tentacles at about the same time. At, or often somewhat before this time, ectodermal disintegration begins at the tip of the manubrium and progresses basipetally over the manubrium and body of the hydranth (Figs. 2 and 3). The short stalk below the hydranth differs in relative susceptibility in different cases, and this difference, as in the case of the 'stalk' of *Hydra* (Child and Hyman, '19) apparently depends on the occurrence or non-occurrence of contraction. In cyanide, particularly the higher concentrations, this stalk usually contracts and in such cases its ectoderm disintegrates at about the same time as that of the mouth region (Fig. 2), but where contraction does not occur, it disintegrates only after ectodermal disintegration of the body is completed (Fig. 3).

In general the entoderm disintegrates later than the ectoderm, except near the mouth region. Since the agent must pass through the ectoderm to reach the entoderm, this difference is perhaps not significant, though it may be noted that in neutral red the entoderm stains almost as rapidly as the ectoderm and may stain much more deeply than the latter before death occurs. In the proximal tentacles the solid column of entodermal cells shows a basipetal susceptibility gradient, like the ectoderm, disintegration beginning at the tip of the entodermal column when ectodermal disintegration has progressed half.way more or less to the base (Fig. 1). In the distal tentacles the difference in susceptibility between ectoderm and entoderm is similar. In the mouth region the entoderm may disintegrate as early as, or sometimes earlier than the ectoderm, perhaps because the agent enters through the mouth to some extent, though, so far as observed, the mouth remains closed until disintegration begins in that region. As regards the rest of the body and the stalk, entodermal disintegration is distinctly later than ectodermal and is usually without any very clearly marked gradient.



Figs. 1–3. Diagrammatic optical sections of *Pennaria* hydranths to show the course of disintegration in lethal concentrations. Fig. 1. Ectoderm of proximal tentacles disintegrated half way to base; entoderm beginning at tips. Ectoderm of terminal knobs of distal tentacles disintegrated. Fig. 2. Ectoderm disintegrated to bases of both series of tentacles; entoderm of proximal tentacles two thirds disintegrated, of distal tentacles half disintegrated. Ectoderm of short stalk below hydranth disintegrating. Fig. 3. Tentacles, hypostome and apical one third of hydranth body completely disintegrated. Ectoderm of hydranth body disintegrated to bases of proximal tentacles. Ectoderm of stalk intact.

Here as in *Hydra* (Child and Hyman, '19, p. 193) the entoderm of the mouth region is apparently much more susceptible than that of other body regions, a difference which is perhaps associated with specially intense glandular activity in this region, of which there is some histological evidence.

The length of time between exposure to the agent and the beginning of disintegration and the rate of its progress may of course be varied widely by varying the concentration. The concentrations most used are those in which disintegration begins in one half to one hour after exposure and is completed in three to six hours.

The susceptibility relations in the well developed hydranth of *Tubularia crocea* and in *Bougainvillea*, except that in the latter there is only one series of tentacles, are similar to those in *Pennaria*. The campanularian hydranths examined also show essentially the same relations in fresh material.

THE DEATH GRADIENTS IN STEMS AND STOLONS.

Data on susceptibility of stems to chemical agents, as measured by disintegrative changes, are of course open, in all hydroids possessing a perisarc, to the possible objection that differences in permeability and thickness of the perisarc at different levels may constitute complicating, or perhaps the chief factors in determining the time of death. The thickness of the perisarc is least in the most recently developed, *i. e.*, in any particular axis, the most apical portions of the stem and its thickness increases in general basipetally.

As a matter of fact, however, the perisarc is apparently penetrated rather readily by many agents. It does not obstruct or retard greatly the entrance of vital dyes and permanganate penetrates it almost at once, even where it is thick. While the possible complicating effect of the perisarc must of course be admitted, various facts cited below indicate clearly enough that it is not responsible for all the differences in susceptibility observed in stems.

In general a stem is less susceptible than the hydranth it bears, and the susceptibility decreases basipetally in the stem, at least near the hydranth, in all agents used in lethal concentration. In concentrations of KNC, neutral red, etc., which do not kill within a few hours, the more apical portions of stems usually undergo resorption and retraction within the perisarc after more or less complete disintegration or resorption of the hydranths. These processes of resorption and retraction of the more apical regions are themselves indications of a differential susceptibility in the stems.

The staining with neutral red may be apparently uniform over many millimeters of stem length, while considerable differences in susceptibility, as indicated by the progress of disintegration and decoloration may appear in the same region. As regards the permeability of the perisarc in different regions to KNC, nothing definite can be said, but the susceptibility gradients in KNC are similar to those in neutral red.

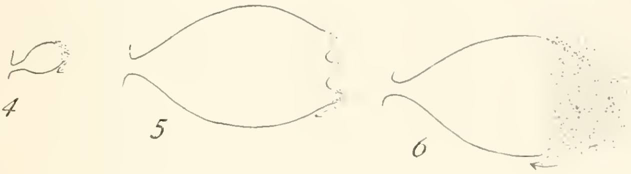
The differences in susceptibility are most marked near the apical ends of stems and stolons, where the differences in rate of growth are greatest within short distances. The growing tip of a stem or a stolon is its most susceptible region, and the susceptibility decreases rapidly from the tip basipetally within a length of several millimeters to a few centimeters, according to the species and the conditions of the stem. In the older regions of stems and stolons differences in susceptibility are much less marked, even over length of several centimeters, and irregularities frequently appear, as in the older portions of the thalli of algæ (Child, '16*a*, '*16b*), some regions dying earlier than regions above or later than regions below. In these older parts susceptibility is often the same in regions where differences in thickness of perisarc are considerable, or it may be different in regions covered by perisarc of equal thickness.

In general the apical region of a growing stem which is to develop into a hydranth is more susceptible than the apical region of a growing stolon of the same colony, but no constant differences have been observed between the older regions of stems and stolons. That these differences between apical ends of stems and stolons represent differences in physiological condition is indicated by the fact, to be more fully discussed in a later paper, that the apical ends of stems can be reversibly transformed into stolons by the action of low concentrations of inhibiting agents.

These data on susceptibility in stems and stolons are in agreement with the facts of observation in that they show the regions of greatest growth activity to be most susceptible to lethal concentrations, and the differences in susceptibility cannot be interpreted wholly in terms of permeability or thickness of perisarc. According to the evidence, the susceptibility relations in the hydroids are very similar to those observed in the multiaxial thalli of algæ (Child, '16*a*, '16*b*, '17), and certain other resemblances will be pointed out below.

The Death Gradients in Developing Medusa Buds.

The elongated, naked medusa buds of *Pennaria* have constituted the chief material for these observations. In the earlier stages of the bud the disintegration of the ectoderm begins at the free, apical end and progresses basipetally (Fig. 4). In later stages, where the tentacular outgrowths have begun to develop, these disintegrate first of all (Fig. 5), and the process then progresses basipetally over the body, as in earlier stages (Fig. 6). The entoderm disintegrates somewhat later than the ectoderm and shows, at least in earlier stages, a basipetal gradient. The apical region of the developing medusa bud of *Pennaria* represents of course the marginal region of the fully grown medusa, the subumbrellar region not being open to the exterior until an advanced stage of development is reached.



Figs. 4–6. Stages of disintegration in Medusa buds of *Pennaria*. Fig. 4. Ectodermal disintegration beginning apically in very early bud. Fig. 5. Later bud stage with disintegration beginning on the tentacular outgrowths. Fig. 6. Later stage of disintegration.

The earlier stages of the gonophores of *Tubularia* likewise show a basipetal susceptibility gradient in the ectoderm and a similar, less distinct gradient in the entoderm. The conditions in the fully developed, free swimming hydromedusae will be considered at another time.

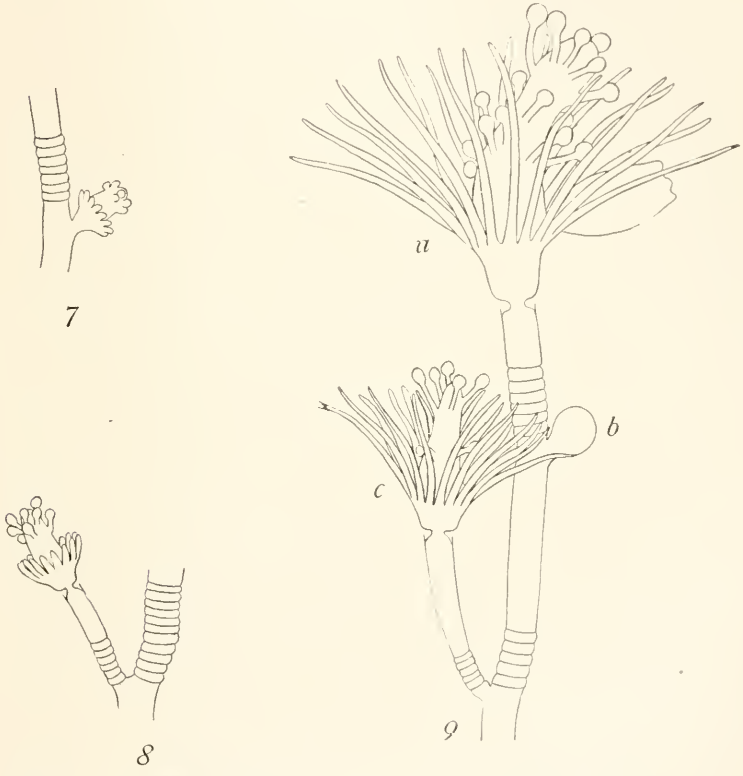
DIFFERENCES IN SUSCEPTIBILITY WITH STAGE OF DEVELOPMENT.

The appearance of motor activity during hydranth development affects susceptibility to some extent as in the case of *Hydra* (Child and Hyman, '19). In general motor activity increases susceptibility, as numerous experiments, not only with *Hydra* and hydroids, but with other forms have shown. The effect of motor activity on susceptibility in *Planaria* has long been used as an experiment in laboratory class work. The earlier stages of hydranth development are non-motile, the later stages motile, *e.g.*, in *Pennaria* the stage of Fig. 7 shows no motor activity, the stage of Fig. 8 is slightly motile and the stages *a* and *c* in Fig. 9 are highly motile. In spite of this difference the early stage of Fig. 7 is distinctly more susceptible than Fig. 9, *a*, but between the stages of Fig. 7, Fig. 8 and Fig. 9, *c* the differences in susceptibility are not very great. In stages after motility is fully developed the susceptibility shows in general a decrease with increasing size of the hydranths on a particular branch or in a particular region of the colony, except that in monopodial forms such as *Pennaria* and *Bougainvillea* certain peculiar conditions exist in the terminal hydranths of the colony and main branches (see p. 114 below).

The effect of motor activity on susceptibility is also evident in the susceptibility of the tentacles, as compared with that of the hydranth body in different developmental stages. In the early stages, *e.g.*, Fig. 7, the tentacles are non-motile and disintegrate at about the same time as the region of the body to which they are attached, but after they develop motility, they are the most susceptible portions of the hydranth, as noted above, and in the forms with two series the proximal tentacles are more susceptible than the distal. In *Pennaria* the proximal tentacles of the advanced stage, Fig. 9, *a*, begin to disintegrate at about the same time or a little later than those of the early stage, Fig. 7, but at this time the body of the early stage is disintegrated down to the level of the proximal tentacles, while the body of stage *a*, Fig. 9 does not begin to disintegrate until later, the actual times depending on concentration of the agent used.

In all other forms examined, *Tubularia*, *Bougainvillea*, the campanularian, *Obelia*, the earlier stages are in general more susceptible than the later, though some differences depending on motor activity and on position in the colony (pp. 112–117) appear.

The earliest stages of bud development (Fig. 9, *a*, Fig. 10, *g*, are, with rare exceptions, less susceptible than naked early hydranths. Since these early stages are enclosed in a thin perisarc they are perhaps not directly comparable as regards susceptibility with later naked stages, but in *Obelia* they are in general less susceptible than later stages such as *b* and *f*, Fig. 10, which are also enclosed in perisarc. On the other hand, they are more susceptible than the stem regions from which they arise. These



FIGS. 7–9. *Pennaria tiarella.* Figs. 7 and 8. Early hydranth stages. Fig. 9. Apical region of a stem, showing monopodial form of branching. The hydranth *a* remains apical, the stem below it increases in length and the bud *b* becomes a lateral branch like *c*.

facts indicate that real physiological differences exist in the different bud stages. Probably the lower susceptibility of the earliest as compared with later stages means that they have not yet attained their maximum physiological activity as regions of growth. The period of early bud development is certainly a period of acceleration in physiological activity and so of increasing susceptibility like the earlier stages of embryonic development (Child, '15*a*, Chap. XV.). Later, as differentiation of the hydranth progresses, susceptibility again decreases.

The sensitiveness of these hydroids to laboratory conditions, as well as the close relation between susceptibility and physiological condition is indicated by the fact that colonies kept over night in standing water or even in aquaria with running water very frequently show, either little or no difference or a more or less complete reversal as regards the susceptibility of different stages of development to KNC, *i.e.*, those stages which were originally most susceptible are either no more susceptible or even less susceptible than other stages. A similar reversal appears when the animals are stained for several hours in neutral red and then killed in KNC. Reversals have also been observed in various algæ under the same conditions (Child, '16*b*, '17). In the hydroids, as in the algæ, it is evident that laboratory conditions or neutral red alter the physiological condition of the earlier, originally more susceptible developmental stages to a greater degree than that of the later stages, and that this alteration decreases the susceptibility of the earlier stages to KNC to such an extent that it is equal to or less than that of later stages. Since susceptibility to KNC shows a close relation to rate of oxidation, it is probable that laboratory conditions and neutral red decrease oxidation in some way and to a greater degree in earlier than in later stages. In other words, this effect of laboratory conditions is itself dependent upon the differences in susceptibility which exist in these organisms. Moreover, this effect and that of neutral red on the one hand, and that of KNC on the other are evidently not additive, as the effect of KNC and lack of oxygen appear to be (Child, '19*b*).

The susceptibility of the medusa bud, like that of the hydranth, decreases with advancing development in all stages preceding the development of motor activity, except the very earliest. In *Pennaria* the earliest visible stages show no distinct difference in susceptibility from the hydranth body on which they arise, but somewhat later stages (Fig. 4) are slightly more susceptible than the hydranth body, though distinctly less susceptible than young hydranths of the stage of Fig. 7. Half grown buds are almost always slightly less susceptible than the hydranth body to which they are attached, and the still later stages are much less susceptible than the hydranth and are the least susceptible structures in the colony except the older parts of the stems.

In *Tubularia* the gonophores, which are modified medusæ, show a decrease in susceptibility from a certain early stage on, and the later stages are less susceptible than the hydranth from which they arise. In *Bougainvillea* also, where the medusæ arise singly from special buds instead of from the hydranth body, the differences in susceptibility with stage of development are the same.

The gonozoöid of the campanularian species examined is in general less susceptible than the fully developed hydranth, and even the earlier stages of gonozoöid development are less susceptible than early hydranth stages, though more susceptible than late hydranths, in spite of the presence of perisarc. The medusa buds on the blastostyle show differences in susceptibility with developmental stage similar to those observed in the medusa bud of *Pennaria*, the advanced stages being much less susceptible than corresponding hydranth stages.

The results with medusa buds of different species are then consistent. The medusa bud represents a reproductive process with a certain amount of dedifferentiation in the earliest stages, and in these stages some increase in susceptibility appears, as compared with the region from which the bud arises, but as differentiation progresses, the susceptibility again decreases. The changes are similar to those in the hydranth, except as the latter are masked by the development of motor activity in relatively early stages. The susceptibility of the medusa bud, however, is in general lower than that of corresponding stages of hydranth development, a fact which agrees well with the greater differentiation of the medusa. In short, the susceptibility relations indicate that the medusa represents physiologically a more advanced stage of development than the fully developed hydranth and is therefore physiologically older and less susceptible.

Colony Gradients.

The hydroid colony as a whole represents a more or less orderly integration of the constituent units. The general growth form or arrangement of zoöids is more or less definite and characteristic for the species, and this definiteness of form and arrangement is particularly characteristic of those species in which a main stem and lateral branches, with perhaps secondary and tertiary branches can be distinguished. In these respects the hydroid colonies resemble multiaxial plants, and such plants are colonial forms in the same sense as the hydroids, *i.e.*, each growing tip in the plant, like each zoöid in the hydroid, represents at least the apical region of a physiological axis, an individual, with more or less complete development of other parts. Moreover, the two general types of relation between the different constituent axes which are characteristic of plants, viz., the monopodial and the sympodial forms of branching, are also found in the hydroids. In *Pennaria* and *Bougainvillea* and various other tubularian hydroids the colony is of the monopodial type, *i.e.*, new axes arise as lateral branches of a persistent main axis (Fig. 9). In *Obelia* and various campanularians examined the colony is sympodial in type. Here each new bud becomes the apical end of the apparent main axis, the former apical zoöid being dis-

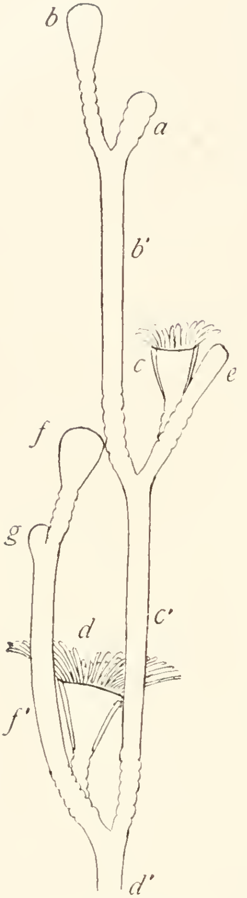


Fig. 10. Apical region of stem of *Obelia borealis* Nutting, to show sympodial method of branching: (*a*) Latest bud on main axis; arose as a lateral bud from the axis *bb'*. The axis *bb'* arose as a lateral bud from the axis *cc'*, and axis *cc'* as lateral bud from axis *dd'*. As the stem of hydranth *d* elongated a second bud appeared and produced the axis *ff'*, and from this axis a new axis *g* is just developing. The stem of hydranth *c* has also produced a second bud which has given rise to the axis *e*. In this way the lateral branches develop.

placed and becoming the tip of a lateral branch (Fig. 10). In these forms what is apparently the main axis is actually made up of a portion of each successive axis formed. In the monopodial *Pennaria* the apical zoöid is, except for injuries or losses, the first zoöid developed and so the oldest in point of time of that stem or branch, while in *Obelia* the apical zoöid is the latest developed and the youngest in point of time, except for the new bud which is soon to take its place.

In both types, however, certain characteristic relations exist between main axes, real or apparent, and lateral branches. In *Pennaria* and the *Obelia* species examined, for example, lateral branches grow more slowly than the main axis, so that the colony developing without injury or crowding shows more or less the general form of a conifer. It has been experimentally determined that in plants of such form very definite physiological relations exist between main and lateral axes, and it has been possible to show for various algæ of these growth forms that the susceptibility relations are likewise very definite in character (Child, '16*a*, *b*, '17). The similarity of growth form in plants and hydroids indicates that the physiological relations between the constituent members of the complex must be in certain respects similar and therefore quite independent of the specific differences in plant and animal protoplasm, in other words quantitative in character.

The susceptibility of the hydranths of different regions of the whole colony or of particular stems or branches shows certain characteristic differences which are similar to those found in the algæ and associated in a definite way with the growth relations of the constituent axes. In a well developed colony of *Pennaria* all primary branches, except those latest formed just below the apical end of the main axis, terminate in large hydranths of about the same developmental stage, so far as appearance is concerned, though of course, where losses and regenerations have not occurred, the apical hydranth of the main axis is the oldest in point of time, that of the most basal branch the next oldest and that of each successive branch successively younger. In fresh material, however, the susceptibility of these full grown terminal hydranths decreases in general basipetally in the colony, *i.e.*, the terminal hydranth of the main axis, although the oldest of all in point of time, and the terminal hydranths of the more apical branches, which are, as regards time, the youngest terminal hydranths, are on the average more susceptible than terminal hydranths of more basal branches. For example, characteristic cases show 60–80 per cent. of the fully developed terminal hydranths of the apical half of a colony disintegrating at a time when 60–80 per cent. of the terminal hydranths of the basal half are still intact. In any well developed primary branch bearing secondary branches, the susceptibility differences in the terminal hydranths of the secondary branches are in general similar to those in the primary terminal hydranths. The terminal hydranth of the primary branch and the hydranths of the more apical secondary branches are on the average more susceptible than those of more basal secondary branches. In short, the colony as a whole and each larger branch shows definite and orderly susceptibility relations among its hydranths, and these relations are similar to those observed in algae with similar growth form. *Bougainvillea* shows similar susceptibility relations, but as might be expected from the less regular form of colony they are less distinct and uniform than in *Pennaria*. It is perhaps necessary to emphasize the point that these are general and average differences and are best determined by comparing percentages of terminal hydranths in process of disintegration at a given time in different regions of the colony, rather than by comparing individual hydranths. The differences are not as great as the differences with stage of development and are clearly brought out only by some statistical method. Even the apical hydranth of the colony does not always disintegrate earlier than any other fully developed hydranth, but it does do so in a large proportion of the cases in fresh material and the relative susceptibility of the other terminal hydranths is likewise not absolutely uniform. In fact this relation in the hydroid is much less distinct and uniform than in many of the algæ.

It is a fact of some interest that in *Pennaria* and *Bougainvillea* the apical hydranth of the colony is the most susceptible or among the most susceptible of the fully developed hydranths, although the oldest in point of time, and that the terminal hydranths of branches differ in susceptibility as noted above, according to their position in the colony. These differences in physiological condition indicate that for some reason the terminal hydranths of the colony and many branches undergo physiological senescence less rapidly than others. In this connection it may be pointed out that the terminal hydranth is more closely associated than any other with the process of hydranth reproduction, since the new hydranth buds arise a short distance below it (Fig. 9). Moreover, growth and the development of new buds occurs most rapidly in the apical region of the main axis of the colony, and the rate of growth and budding decreases basipetally in the colony as a whole and in each branch. Apparently, as in those plants with similar growth form, there is a relation of dominance and subordination in the colony and in each branch, the apical region being dominant and inhibiting growth and reproduction in other axes to a greater or less degree.

Since new buds arise a short distance below the apical hydranth of any axis (Fig. 9), they probably produce some effect upon the hydranth, either in the way of reorganization or through the demand for nutrition which they create. The nutrition for the bud must be derived largely from the reserves or the newly digested food of the apical hydranth, consequently the nutritive condition of this hydranth may be altered to some degree in the direction of starvation, but when the new hydranth becomes able to obtain food for itself the apical hydranth may recover its former nutritive condition. The effect of starvation in producing regression to a physiologically younger condition in the lower invertebrates has been clearly demonstrated (Child, '14, '15*a*, Chap. VII, '19*a*) and it seems probable that alternating changes of this sort associated with the development of new buds are concerned in the retardation of senescence in the apical hydranths. In a monopodial colony such as *Pennaria* the hydranth in which such changes occur most frequently, *i.e.*, the apical hydranth of the colony, will, according to this hypothesis, undergo senescence least rapidly, and the relative rate of senescence in the apical hydranth of any axis will vary in general inversely as rate of growth and frequency of reproduction in that axis. Consequently, since rate of growth and frequency of reproduction decrease basally in the colony and its branches, the more basal hydranths and regions undergo senescence more rapidly.

The whole complex of physiological relations in the colony represented by the differences in rate of growth, reproduction and susceptibility in different regions results from the order of development. The colony begins as a single axis terminated by a hydranth. When the stem attains a certain length a bud appears at a certain distance below the terminal hydranth and is probably inhibited to some extent by it. As growth of the main stem continues, a second bud arises above the first and now the earlier bud or hydranth is subjected to the inhibiting action of two apical regions, and so on as other buds develop. The same relations arise in each branch as it develops. It follows that in any axial complex the degree of inhibition will increase basipetally.

In *Obelia* and other sympodial forms the situation differs in one respect from that in the monopodial *Pennaria*. The apical hydranth of the colony and of each branch in *Obelia* is the latest developed, the youngest both in point of time and physiologically of the axial complex which it terminates. In well developed colonies of *Obelia*—colonies of *O. borealis* 10–14 cm. in length and very symmetrical in general form are common at Friday Harbor—a general apico-basal gradation in susceptibility of these young apical hydranths or buds of different primary branches appears, those of more apical branches of the colony being somewhat more susceptible than those of more basal branches, like the terminal hydranths of *Pennaria*. Similar differences appear to some degree in the secondary branches of a primary branch. Moreover, the same differences in rate of growth and reproduction as in *Pennaria* appear in different branches from apex to base of the colony or in the secondary branches of any branch, in other words the same relations of dominance and subordination exist in the two growth forms as wholes.

The chief difference between the two forms consists in the fact that in the monopodial *Pennaria* the terminal hydranth maintains its dominance over later buds and therefore remains terminal, while in the sympodial *Obelia* the latest bud becomes dominant and displaces the former terminal hydranth. In this connection it is of interest to note that the *Obelia* bud grows for some time before it differentiates into a hydranth (Fig. 10). During this period of growth it is the seat of intense physiological activity and shows a high susceptibility, which decreases as the hydranth develops, and it is during this period that it displaces the former apical hydranth. In *Pennaria* the bud differentiates into a hydranth at once with but little growth in length (Fig. 7). That these differences in behavior are associated in some way with the differences in growth form cannot be doubted, and it seems probable that they may be the determining factor. Since dominance in axial relations has been shown to be fundamentally associated with differences is intensity of metabolic activity the intense growth activity of the bud of *Obelia* and similar forms probably enables it to dominate and displace the former apical bud which has completed its period of rapid growth and is undergoing differentiation into a hydranth. In *Pennaria*, on the other hand, where the bud differentiates with very little growth, the intensity of its activity decreases from an early stage and is apparently less at any stage than in the *Obelia* bud, consequently it is unable to alter existing axial relations and becomes a subordinate member of the complex. This interpretation is offered merely as a suggestion, based on the data at hand. Whether it is correct or not can probably be determined only by experimental work such as has been done with plants on control and modification of the relations of dominance and subordination by inhibition or removal of certain apical regions, but such work offers difficulties at present.

The necessity of using freshly collected material for the study of the regional differences in susceptibility, the colony gradients, as well as for other work on susceptibility may once more be emphasized. In colonies kept in the laboratory over night the regional differences have often disappeared or are reversed in the colony or branch.

That there is a real difference in physiological condition in different regions of the colony is indicated by the fact that the medusa buds in *Pennaria* and *Bougainvillea* and the gonozoöids in *Obelia* appear first in the most basal regions of the most basal branches, *i.e.*, the regions where the susceptibility is on the average lower than anywhere else in the colony. From this region the localization of developing medusa buds and gonozoöids gradually progresses apically in the colony as a whole and in each branch. Moreover, at least many hydranths of *Pennaria*, after producing medusa buds for a time, degenerate and may be replaced by new ones, but whether such degeneration always follows medusa bud-formation, it is impossible to say. In *Obelia* the hydranths, which in this form have no direct part in the production of medusae, undergo disintegration or resorption, at least very frequently in regions of the colony basal to the level of gonozoöid formation. New hydranths may develop in place of the old, so that this loss of hydranths in relation to gonozoöid formation may readily escape notice, unless carefully looked for.

Evidently the physiological condition which determines the development of medusa buds and gonozoöids is attained in the basal regions of the colony earlier than elsewhere, and gradually progresses apically in the colony, followed by a zone in which at least many of the hydranths die or undergo resorption. This region where medusa buds or gonozoöids make their first appearance is the region where rate of growth and frequency of hydranth buds has been least and therefore physiological senescence has progressed most rapidly. In the sympodial colony of *Obelia* these regions are the oldest in time as well as physiologically, while in the monopodial *Pennaria* and *Bougainvillea* they are the oldest in time except the apical hydranths, but physiologically are the oldest of all, apparently because senescence has been retarded in the apical hydranths by the higher frequency of hydranth reproduction. There can be no doubt that the appearance of medusa buds or gonozoöids in a colony is associated with the attainment of a certain stage of progressive development and physiological senescence, and the facts presented above, with their interpretation, throw some light on the problem of this first appearance in basal regions and gradual progression apically in the colony.

In various algæ of similar growth form to the hydroids the specialized reproductive organs, *e.g.*, the cystocarps in numerous species, the stichidia in *Dasya* and related forms, make their first appearance in the basal regions of the thallus and the zone of their development progresses apically, exactly as in the hydroids. Moreover, in the algæ as in the hydroids, the basal regions are the regions of lowest susceptibility and the oldest physiologically (Child, '16 *a*, *b*). That this similarity of localization of specialized reproductive parts, as well as the similarity of growth form in algæ and hydroids is associated with the gradations in physiological condition, of which the susceptibility relations are an indicator, cannot be doubted. And finally, it may be pointed out once more that this similarity in growth form, susceptibility relations and localization of specialized reproductive processes in algæ and many other plants and hydroids must depend upon some physiological factor which is independent of the specific differences in protoplasmic constitution of the different forms and which must therefore be quantitative in character. In short, all the facts force us to recognize the existence of a quantitative gradation in physiological condition, in rate of fundamental metabolism and the associated protoplasmic conditions, as the basis of the axial relations, of which growth form, susceptibility and localization of parts are various expressions.

Concerning the nature of the relation of dominance and subordination which has long been known to exist in the plants and is evidently present in the hydroids also, it need only be said here that the conception of dominance by means of inhibiting, formative or other specific substances accounts neither for the origin nor for the maintenance of these relations. In order that one region may produce substances that inhibit another region, the two regions must already be different, *i.e.*, in order that such chemical relations may exist along a physiological axis, the axis must already be established and certain differences be present. Moreover, if one apical region produces substances which inhibit other apical regions, the one region must be immune to the substances which it produces, while the other regions are not, that is to say differences must already exist between the different apical regions before one can inhibit another without itself being inhibited, and if differences already exist, the assumption of the existence of inhibiting substances becomes wholly unnecessary. And finally, the definite and characteristic spatial relations between different axes in such a complex as the multiaxial plant and the hydroid cannot be accounted for in terms of the production and transportation of inhibiting or other substances. No sort of transportative relation can be conceived which affords an adequate interpretation of the facts. Only on the basis of transmission in protoplasm is it possible to account for both origin and maintenance of the relations which are demonstrated to be present in the organism. Of course after parts have become qualitatively different, more or less specific chemical relations undoubtedly exist between them, but these are results of physiological integration, not its determining conditions. Further discussion is postponed until additional evidence along other lines is presented.

Gradients in Staining with Vital Dyes.

When neutral red or methylene blue is used in determining differences in susceptibility, it is found that while these dyes enter all cells of the hydranth readily, differences in rate of staining appear in the earlier stages of the process. These differences show the same relations to the axes and to stage of development as the differences in susceptibility. The axial gradients in rate of staining indicate the existence either of differences in permeability to the dyes or of differences in the rate at which they are adsorbed or otherwise taken up by the cell constituents, or perhaps differences of both sorts. That the dyes are held in some way within the cells is evident from the fact that they accumulate there until their concentration is far higher than outside and do not pass out to an appreciable degree when the animals are returned to water. Similar differences in rate of staining with vital dyes, showing in general the same regional relations as the differences in susceptibility, have been noted in many other forms, both plant and animal, but it is evident that in the hydroids, as elsewhere, the differences in susceptibility to the dyes, as measured by time of death, cannot be due alone to the fact that the dyes enter certain cells more rapidly than others. Most of these differences in rate of staining, *e.g.*, those in different parts of the hydranth are slight or only temporarily visible in the earlier stages of staining, but the more extreme differences, such as those between hydranths and stems, remain visible for a longer time, in fact the hydranths may be killed by the stain before the stem is very deeply stained. In general, however, the differences which appear at first tend to become equalized as staining progresses. Apparently the rate of accumulation of the dye decreases most rapidly in those cells which at first stain most rapidly, so that the early differences in rate of staining are obliterated, at least to a large extent, by the action of the dye on the cells.

Nevertheless the differences in susceptibility, as indicated by survival time are very marked in these dyes, even in regions where the staining appears to be uniform in degree. The early differences in rate of staining are evidently not alone responsible for the differences in susceptibility. In fact it is much more probable, as has been suggested elsewhere (Child, '16*b*, Child and Hyman, '19), that the conditions indicated by the differences in staining rate, whether differences in permeability, adsorption or some other factor, are simply one aspect of the differences in fundamental physiological condition which determine differences in susceptibility. The facts of acclimation briefly referred to below, so far as they concern the vital dyes, show even more clearly that the differences in physiological effect are not due to the initial differences in rate of staining, for parts which stain at first more rapidly show a greater capacity for acclimation than less rapidly staining parts.

As death approaches in cells stained with neutral red, a distinct change in the color of the dye toward the acid side occurs, *i.e.*, the cell contents become distinctly acid shortly before disintegration. This change in color of neutral red occurs quite independently of the nature of the killing agent, which may, for example, be neutral red itself, an acid, KNC which is alkaline in solution, etc. Of course as soon as disintegration occurs the color of the neutral red is determined by the hydrogen ion concentration of the entering solution. The increase in acidity preceding death is sufficient to be clearly visible and in elongated axiate parts, *e.g.*, the tentacle or hydranth body, a gradient in acidity preceeds the disintegration gradient. Similar increase in acidity preceding death has been observed in the cells of many algæ (Child, '16 *a*, *b*, '17). In many species of algæ this gradient in acidity is distinct within the length of a single elongated cell, even though the staining may have been quite uniform up to this stage. The axial relations of this increase in acidity are of course the same as those of the disintegrative changes and the color change of the dye may itself be used to a considerable extent as a criterion of susceptibility.

ACCLIMATION.

Presentation of certain data which bear more or less directly upon the problem of acclimation, *e.g.*, the resorption and redevelopment of hydranths, the transformation of apical regions from hydranth production to stolon production and the reverse, must be deferred to another paper. For the present it may be noted that in low concentrations of various agents, KNC *m*/50,000–*m*/20,000, HCl *m*/2,000–*m*/1,500, $LiCl$ *m*/50, ethyl urethane *m*/2,000–*m*/500, $MgSO_4$ *m*/500, neutral red and methylene blue, indications of differential acclimation appear. In the single hydranth under such conditions the body may die before the tentacles, but in most hydroids the hydranths undergo either disintegration or resorption so rapidly under slightly depressing conditions in the laboratory that the investigation of differential acclimation in the single hydranth is difficult.

Attention has been chiefly directed, however, to the regional differences in acclimation in the colony as a whole, which are very definite and characteristic. In general, the physiologically older hydranth shows less capacity than the younger hydranth or the bud for acclimation to slight inhibitions, consequently, in the course of a few days under such conditions, the older hydranths disintegrate or undergo resorption, while the younger persist or are affected to a less degree or less rapidly. Since the order of arrangement of hydranths of different physiological age along each axis and in the colony as a whole is definite and characteristic for the species (pp. 112–117), the regional differences in acclimation to low concentrations are in general the same as the differences in susceptibility to lethal concentrations, that is, those zoöids which are most susceptible to the lethal concentrations, show the greatest capacity for acclimation to a certain range of low concentrations.

Conclusion and Summary.

The significant points in the experimental data are; first, that the susceptibility relations are definite, not merely for the single tentacle, hydranth and medusa bud, but for the single axis as a whole and for the axial complexes which result from branching and finally for the whole colony; second that they are similar to the relations observed in other organisms with similar growth forms, *e.g.*, the algæ. In general, the more apical regions of the physiologically younger axes of a complex, which are obviously the regions of most intense metabolism, are most susceptible to the higher concentrations or intensities and also most capable of becoming acclimated to slightly inhibiting conditions. These differences in susceptibility and capacity for acclimation are manifestly associated with quantitative differences in physiological condition, which are fundamental characteristics of physiological axes. The similarity of susceptibility relations in algæ and hydroids of similar growth form, indicates, if it does not demonstrate their independence of the specific differences in algal and hydroid protoplasms and therefore their essentially quantitative character. In spite of the differences in rate of staining with vital dyes, it is evident that differences in susceptibility cannot depend solely upon differences in permeability of limiting surfaces, though such differences in permeability are doubtless associated with the conditions on which susceptibility depends. Regional or axial differences in permeability are themselves dependent upon the fact that the membranes are living and therefore metabolically active, and upon the possibility of quantitative differences in this living condition. Some substances enter cells only as they produce irreversible changes in the living surface, while others enter all cells readily without appreciable injury to the surfaces, yet to both groups of substances the axial and regional differences in susceptibility are in general similar.

These data on susceptibility in the hydroids are in complete agreement with other data on both animals and plants and constitute another bit of evidence in support of the conclusion that physiological axes are primarily gradients in rate of fundamental dynamic processes and in associated physical and structural conditions in living protoplasm.

The most important facts are summarized as follows:

1. In the hydranths of *Pennaria*, *Tubularia*, *Bougainvillea*, *Obelia*, *Gonothyria* and various other forms, the susceptibility to lethal concentrations of at least many external agents is greatest in the apical region of each tentacle and of the hydranth body and decreases basipetally.

2. In the elongated medusa bud of *Pennaria* the susceptibility decreases basipetally from the apical region.

3. Stems are in general much less susceptible than the more highly specialized structures, though the perisarc may act as a protective covering to some degree. At least in the more apical regions of axes the susceptibility of the stem also decreases basipetally, but in the more basal regions irregularities may appear.

4. Physiologically older zoöids or medusa buds are less susceptible than younger, except in certain cases where the development of motility increases susceptibility to such an extent as to overbalance the age differences.

5. Except in the earlier stages the medusa buds of *Pennaria* are less susceptible to lethal concentrations than the hydranth bodies from which they arise, and the gonophores and gonozoöids of other forms show the same relations.

6. In the colony as a whole and in each axial complex the average susceptibility of well developed hydranths decreases basipetally, but certain characteristic differences in axial relations exist in the monopodial (*Pennaria*) and the sympodial type (*Obelia*).

7. The medusa buds of *Pennaria* and *Bougainvillea* and the gonozoöids of *Obelia*, etc., appear first on those hydranths or in those regions of the colony where the susceptibility to lethal concentrations of agents is least, *i.e.*, in the basal regions of the more basal branches. From these regions the zone of development of these parts gradually progresses apically as the different levels successively attain the physiological condition which determines their development.

8. The very close relation between susceptibility and physiological condition is indicated by the fact that in various species the characteristic gradients and regional differences in susceptibility often disappear or are reversed after a day or two, or in some cases, after a few hours under laboratory conditions.

9. In general the regions which are more susceptible to lethal concentrations are more capable of acclimation to a certain range of lower concentrations.

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LOCOMOTION IN TWO SPECIES OF THE GASTROPOD GENUS ALECTRION WITH OBSERVATIONS ON THE BEHAVIOR OF PEDAL CILIA.

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Introduction.

In a paper on locomotion in gastropods Parker ('11) has pointed out that the mud snail, *Alectrion* (*Ilyanassa*) *obsoleta* Say, exhibits no rhythmic pedal movements such as are readily observed in most snails, and concludes that progression is accomplished by arhythmic muscular activity. He writes: "The movement of the foot of *Ilyanassa* has a most striking resemblance to that of the foot of a planarian in which cilia may be the chief motor organs, but on testing the foot of *Ilyanassa* with carmine suspended in sea water, not the least evidence of cilia could be discovered." In subsequent accounts of gastropod locomotion *Alectrion obsoleta* has been cited as representing the only definitely known case of arhythmic movement. Recently, however, Crozier ('19) has reported another example, that of *Conus agassizii* from Bermuda.

In the course of studies on the reactions of *Alectrion* I became interested in the locomotion of the animal and tested the foot for cilia by dropping carmine grains on its ventral surface. Much to my surprise the carmine was carried down the foot, and when the latter was examined under the microscope the under surface was found to be covered with cilia. Another related species, *Alectrion* (*Tritia*) *trivittata* Say, which showed no evidence of pedal waves likewise proved to have a ciliated foot.

That cilia occur on the feet of certain species of gastropods has been known for some time, but that they function as locomotor organs, however, has been questioned, and I believe that no detailed study of their behavior has been made. Olmsted ('17) has recently reported finding cilia on the feet of three species of marine snails from Bermuda, which showed no evidence of pedal waves, and attributes their locomotion to ciliary action. Since, however, it is held that progression may be brought about by muscular activity without the appearance of pedal waves (arhythmic locomotion), the presence of cilia and the absence of waves does not signify necessarily that locomotion is accomplished by cilia. It is conceivable that pedal cilia have some other function, such as freeing the foot from foreign material or distributing mucus. If some such conception of ciliary function had not been held by students of gastropods, movement by ciliary action should have been recognized long ago as unquestionably constituting one type of locomotion in these animals. Walter in 1906 described the mechanism of locomotion in the pond snail, *Lymnæus elodes* Say, as consisting of "a complex muscular foot clothed on the ventral surface with cilia which act on a mucus track," and did not record finding any pedal waves, yet this case, as far as I know, has never been cited as substantiating the theory that gastropod locomotion may sometimes be effected by cilia.

In view of these facts it seemed desirable to investigate in some detail the behavior of the cilia on the feet of *Alectrion* in the hope of throwing light upon the possible relation between ciliary action and locomotion. The work was carried on at the Marine Biological Laboratory at Woods Hole, and I wish to express my gratitude to the director, Dr. Frank R. Lillie, for his kindness in granting me the facilities of the laboratory.

CILIARY BEHAVIOR.

Alectrion trivittata.—The foot of *Alectrion trivittata* in an individual of medium size measures 10 or 12 mm. in length by 4 or 5 mm. in width. The anterior end is truncated and auriculate. Back of the lateral, pointed processes the foot is narrowed somewhat before it expands and tapers toward the posterior end, which is bifurcated and bears two small tentacles. It is more slender than the foot of *Alectrion obsoleta* and considerably thinner, a feature which makes the species a favorable one for the study of ciliary activity under the microscope.

As soon as it was recognized that the under surface of the foot was ciliated the question arose: Were the cilia active during locomotion, and if so, when the animal came to rest, did ciliary movement stop, or did it continue without effecting forward movement? That the cilia did not beat continuously was soon demonstrated. A support for the snail was made by fastening with "sticky-wax" a short glass tube to the bottom of a glass dish containing sea water. The snail's shell was then stuck upside down to the end of the pedestal so that the ventral surface of the foot when fully expanded was at the level of the water. In this position the animal usually exhibited righting movements for a time, but soon spread out its foot on the surface film of the water. Often the flicker of the cilia could be seen with the naked eye and readily observed under a hand lens. Grains of carmine were swept down the foot by ciliary activity. At other times, however, the cilia were quiescent and carmine grains remained on the foot where they were dropped. When a snail resting in this position was stimulated by touching one of its tentacles with a piece of fish meat, the proboscis was protruded and the cilia began beating. The proboscis was often worked over the surface of the foot and at that time the ciliary movement was greatly reduced or stopped. Usually after the proboscis was withdrawn the cilia showed vigorous activity, the foot was stretched out its full length, and the animal appeared as it did when moving upside down on the surface film of the water, a form of progression often exhibited when free in the aquarium. In the later situation its behavior was essentially like that when fixed in the position just described. As it moved along the surface of the water the cilia could be seen in motion and carmine grains were swept with the mucus down the foot. Frequently the proboscis was extended and moved about in various directions, and at this time ciliary activity and locomotion ceased.

By placing the dish, in which an inverted snail was fastened, on the stage of a microscope, and by using a low power objective the fringe of long cilia projecting from the anterior border of the foot could be clearly observed. In one instance when the animal was becoming quiet after feeding, the cilia could be seen beating slowly near the tip of one of the anterior lateral foot processes, whereas other cilia nearer the median position were motionless. Moving cilia were observed slowing down and coming to rest, and passive ones becoming slightly active. Suddenly all the cilia under observation began to beat at high speed, when compared with the slower movement first recorded, and the foot process moved out of the field of vision. The snail was now showing its normal surface "swimming" activity.

The relation of ciliary movement to locomotion was finally studied by observing under the microscope snails which were moving on the under side of a glass slide placed across a dish full of sea water. A resting animal showed the cilia on the anterior border and ventral surface of the foot passive. Sometimes, when the snail was feeding, one of the lateral foot processes was moved or contracted slightly and some local beating of the cilia of short duration occurred. When the animal was moving, all the cilia which could be seen were active, and moreover, the rate at which they beat was distinctly correlated with the speed of locomotion. Rapidly beating cilia decreased their rate of movement and came to rest when the animal did, and became active again when locomotion began. Very satisfactory observations were made by placing the snails on the under surface of a slide, which had been smeared with fish meat, and allowing them to feed in this inverted position out of the water. Under these conditions they frequently progressed short distances and stopped, and the cilia in turn were seen to start beating, to continue slowly while the animal moved, and then to come to rest. When the rate of locomotion was increased, the cilia beat so rapidly that they could only with difficulty be seen at all.

A microscopic examination of the cilia of snails moving and floating at the surface of the water confirmed the observations already reported.

Alectrion obsoleta.—The foot of *Alectrion obsoleta* resembles that of *Alectrion trivittata* but is thicker, not so long in proportion to its width, rounded at the posterior end and lacks posterior tentacles. The behavior of the cilia were studied by some of the methods described in connection with the work on *Alectrion trivittata*, and the results obtained were so closely similar it is unnecessary to report them in detail. The thicker foot of the mud snail made microscopic observation of the cilia on the ventral surface more difficult, but they could be seen when beating, and those along the anterior border were clearly visible at all times.

A comparison of the habits of the two species brought out one striking difference. Whereas *Alectrion trivittata* often floats or moves upside down at the surface of the water after the manner of certain fresh-water gastropods, *Alectrion obsoleta* was never observed to do so. Dimon ('05) in her monograph on the latter species states that young individuals may float on the surface but that the older ones very rarely do, and then only under unusual circumstances, and concludes that the shell of the adult is too heavy to permit it. It was impossible therefore to observe the behavior of the cilia on the foot of a freely floating mud snail.

The study of the two species of *Alectrion* shows unquestionably that beating cilia are characteristic of a moving snail, and that when the animal is at rest the cilia are motionless or exhibit only local activity. It is equally clear that the rate of ciliary movement is correlated with that of locomotion.

The reason why Parker, in the investigation referred to, failed to find cilia on the foot of *Alectrion obsoleta* was undoubtably because when he placed carmine on them they happened to be quiescent. Only the unusual behavior of the cilia prevented their immediate discovery by the test he applied.

Comparison of Locomotor with Ciliary Rates.

The foregoing observations are strongly in favor of the view that progression in *Alectrion* is accomplished by ciliary action. In order to test these conclusions further, the rate of locomotion was compared with the rate at which grains of carmine were driven over the surface of the foot by the beating cilia. The latter may be termed the ciliary rate. By the use of a stop-watch it was an easy matter to secure a series of records indicating the speed at which the snails traveled a distance of 5 mm. over the bottom of a glass dish containing sea water. In order to obtain a ciliary rate, the snail was fixed upside down on a support so that its foot when expanded was at the surface of the water.

A millimeter rule was fastened above the foot. Particles of dry carmine were then dropped on the water and as they were driven with the mucus down the foot by the active cilia, it was possible to record their speed over a distance of 5 mm. with considerable accuracy.

For some time all attempts at securing the ciliary rate for *Alectrion obsoleta* were unsuccessful. The snails either continued in their efforts to right themselves or else failed to expand their feet at the surface of the water, as *Alectrion trivittata* did with little hesitancy. When about to abandon the experiment some individuals not fully grown were tried, and their behavior was so satisfactory the desired records were readily obtained. In one instance continuous ciliary movement was watched for about three quarters of an hour. It is quite possible that the difficulty encountered with the older individuals was due to the fact that in the adult state the species does not have the habit of surface floating, whereas the younger animals adapted themselves to the inverted position more readily because they had more recently abandoned it under natural conditions.

The results of the tests made upon *Alectrion obsoleta* are shown in Table I. They indicate that the average locomotor rate is practically identical with the rate at which carmine grains are driven along the foot by the beating cilia.

TABLE I.

Alectrion obsoleta.

	Locomotor Rate. (Rate at which Snails Moved a Distance of 5 Mm.).			*Ciliary Rate.* (Rate at which Carmine Grains were Carried a Distance of 5 Mm. Over Ciliated Foot.)	
Animal numbe	1	2	6 individuals	1	2
Number of trials	25	25	10 each	25	25
Average time in seconds	2.4	2.9	3.	2.5	3.

Animal number one proved to be a faster moving individual than any of the others timed, and it will be noted that the average ciliary rate is also fast. Considering the fact that the cilia are subject to great variation in rapidity of movement, the correspondence in rates is closer than might be expected, and to my mind affords conclusive evidence that locomotion is entirely dependent on the action of cilia.

Turning now to Table II it will be seen that the average rate of locomotion of *Alectrion trivittata* is twice as fast as that of the mud snail. If one watches for a moment the two species moving about in an aquarium, he does not require the use of a stopwatch to be convinced of this difference. The average ciliary rate of *Alectrion trivittata* (2.3 seconds in eighty trials) is also faster than that of the mud snail, but slower than its own locomotor rate. The latter conclusion is based on many more trials than are recorded in the table. Does this mean that some arhythmic muscular operation is associated with ciliary movement and is responsible for the difference between the two rates, or do the cilia tend to beat faster when the snail is moving on its foot than they do when it is fastened on its back? An investigation of the locomotion of small pieces of the foot led me to believe that the latter explanation is the true one.

Table II.

Alectrion trivittata.

	Locomotor Rate. (Rate at which Snails Moved a Distance of 5 Mm.).			*Ciliary Rate.* (Rate at which Carmine Grains were Carried a Distance of 5 Mm. Over Ciliated Foot.)	
Animal number	1	2	6 individuals	1	2
Number of trials	10	10	10 each	10	10
Average time in seconds	1.8	1.3	1.5	2.7	2.1

The behavior of the cilia on an excised foot will be described later in detail, and it is only necessary to record here that fragments of the foot a few millimeters long move at times rapidly over the substrate by ciliary action. The direction of their movements, however, was so uncertain it was necessary to time their speed over a distance of 2.5 mm. Some of these pedal fragments frequently moved at the rate of 5 mm. in 1.6 seconds and occasionally in 1.2 seconds, or faster than the average rate of locomotion recorded for six snails in Table II. In the case of these small pieces of the snail's foot there was no possibility that locomotion was in any direct way brought about by muscular movements. It was entirely the result of ciliary action, and the resultant speed was closely similar to the usual locomotor rate of the species. Under the microscope the cilia on the fragments were sometimes seen at rest, again beating slowly and frequently at a high rate of speed, all accounting perfectly for the erratic movements of the fragments. There is no evidence that the locomotor mechanism of *Alectrion trivittata* contains any important factor not represented in that of *Alectrion obsoleta*, and I believe that progression in both species is accomplished by cilia.

If, as it appears, the rapidity of ciliary movement in *Alectrion trivittata* tends to be reduced when the animal is held with the ventral side of its foot at the surface of the water, some explanation of this peculiarity should be sought. I believe it is to be found in its habit of surface floating and moving, one not shared by adult mud snails. Observation of the snails' movements at the surface of the water gives one the immediate impression that this form of activity is a decidedly leisurely one associated with feeding. A snail may remain floating with extended proboscis for a long time, and when it moves, it is often for short distances in an apparently aimless way. At such times the cilia probably beat more slowly than they do when the animal is moving in a definite course over a substrate, as it was when the locomotor rates were taken.[1] When the snail is fixed to a support with its expanded foot at the surface of the water, its normal floating position is closely simulated. It accepts the inverted position often without showing any extended disturbance, and always exhibits the feeding reaction when stimulated with fish meat. It would not be surprising, therefore, if the behavior of the cilia were similar under these two conditions, and I believe that a somewhat reduced ciliary activity is characteristic of both.

Ciliary Control.

In connection with the investigations described in the preceding pages there developed the exceedingly interesting problem of ciliary control. Doubtless it has occurred to the reader that the behavior of the pedal cilia of *Alectrion* suggests that they have in some way been appropriated by the nervous system and are controlled directly or indirectly by nervous impulses. Although little more than a beginning has been made in the study of this phase of the problem, the results of certain experiments are sufficiently important to warrant a preliminary report at this time. They have been obtained for the most part from a study of ciliary behavior after the foot has been removed from the rest of the snail's body.

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[1] That snails move more slowly at the surface of the water than they do over a glass surface is perfectly clear, the average speed of locomotion in the former situation, based on thirty-four trials, being at the rate of 5 mm. in 2.7 seconds. Drawing conclusions, however, on the rapidity of ciliary movement by direct comparison of these two locomotor rates may not be justified, for a substrate is not present in both cases.

When the somewhat contracted, excised foot of *Alectrion trivittata* is placed in sea water and viewed under a microscope the greater number of cilia are seen to be motionless. If now the anterior margin of the foot is watched, it will be observed that at intervals slight contractions of the border occur accompanied by the beating of the anterior fringe of cilia over the area moved. This muscular movement is not extensive, but consists of a sudden withdrawal of a short segment of the pedal border so that for a moment it appears slightly indented, the contraction being immediately followed by an expansion which restores the normal contour of the foot. The ciliary activity is also but a momentary affair and is restricted to the contracted region. The cilia usually appear to beat either synchronously with the contraction or directly after it, but in certain instances ciliary movement seems to precede slightly the muscular movement. These contractions accompanied by local ciliary action sometimes continue for hours. Sooner or later, however, the twitching of the muscles becomes more vigorous and frequent, and the cilia fail to come to rest between contractions. Some of them may be beating continuously at the same time that others are still showing intermittent activity associated with muscle movements. Finally the cilia are in motion, not only along the entire anterior border and lateral processes of the foot, but also over the ventral surface, and in the latter position contractions are also going on. At this time the anterior cilia exhibit great variation in rapidity of movement. Occasionally they slow down nearly coming to rest in the intervals between contractions, and at other times move so fast that not the slightest flicker can be seen, the ciliary fringe appearing like a broad hyaline membrane. This high rate of movement is always associated with muscular contractions. Why it was that *Alectrion trivittata* sometimes moved so swiftly was never fully understood until I had witnessed in this way the possibilities of ciliary motion.

The third stage of ciliary activity is seen as a rule a number of hours later, often about twenty-four hours after the foot is severed from the body. At this time all muscular movement has ceased and the whole foot has become expanded. The cilia are beating everywhere at moderate speed, and if a given area is watched, no variation in the rapidity of movement is observed. The contrast between this uniform rate of ciliary movement and the preceding highly variable one is very striking. The cilia continue beating until maceration sets in and the epithelium begins to break down.

Having determined from a series of tests that the ciliary activities described above occurred with remarkable constancy, a means was sought of controlling muscular contractions without directly affecting the cilia. Previous experience had shown that magnesium sulphate is an excellent anæsthetic for marine gastropods and one that appears to leave no undesirable effects. It proved to be equally valuable in the study of ciliary behavior. When a foot of *Alectrion trivittata* in which muscular movements are going on is placed in a ten per cent. solution of magnesium sulphate made up in sea water, all contractions cease in a few minutes and the foot remains passive. The cilia, however, which are vigorously beating when muscular activity subsides, continue to do so for many hours with only gradually decreasing speed, whereas those which are quiescent remain so. Moreover, if there is initial variation in the rate of ciliary movement in different parts of the anterior border of the foot, it is maintained, and the cilia beating the fastest remain active the longest time; but changes in the speed of ciliary movement over a given area, which are so striking when the foot is in sea water and muscular contractions are in progress, never occur after contractility is inhibited by magnesium sulphate. It is also clear that those cilia which are moving most rapidly, or with the least interruption, when the foot is in sea water tend to beat the fastest when the foot is removed to a solution of magnesium sulphate. If now the foot is returned to sea water, muscular contractions reappear in a few minutes and ciliary behavior is just the same as it was before the transfer.

These experiments indicate that the pedal cilia are normally set into activity and their rate of beating is modified either by the contraction of underlying muscle fibers or by direct nervous impulses. The fact that cilia continue beating in a magnesium sulphate solution after muscle activity has disappeared shows too that they have the power like ordinary cilia of maintaining movement in the absence of extra-epithelial stimulation, and finally, since their movement persists until they become exhausted or the epithelium breaks down, it is evident that they depend on some extrinsic factor to bring them to rest. In this connection it should be recalled that a foot which has been in sea water for a long time, perhaps for twenty-four hours, shows the cilia everywhere moving at uniform speed over a given area in the complete absence of muscular movement. In the latter case all the cilia were stimulated to movement before nervous impulses and muscular contractility gradually disappeared, and their behavior is the same as it is after these activities are suddenly inhibited by magnesium sulphate. When a foot which exhibits no muscular contractions is placed in a ten per cent. solution of magnesium sulphate the most marked change noted is but a slight retardation in the rate of ciliary movement. A more concentrated solution may bring about a decided slowing down or even a cessation of ciliary action. This appears to be a direct effect upon the ciliated cells, which show normal activity again after the foot has been transferred to sea water.

During the course of one experiment I was surprised to note certain cilia beating in the magnesium sulphate solution which were quiescent after the foot was placed in the solution twenty-four hours previously. An examination of the foot showed that it had revived during its long immersion in the fluid, and that contractions, such as ordinarily accompany the movement of the cilia, were in progress. Another instance of the reoccurrence of muscular and ciliary activity was recorded in the case of a foot which had remained in a magnesium sulphate solution over twenty hours.

A few experiments were carried on in which the snail itself was placed in a solution of magnesium sulphate. The behavior of the cilia was essentially the same as it was when the foot was severed from the more dorsal parts of the body.

Although the ciliary activities on the excised foot of the mud snail were not studied so thoroughly as in the case of *Alectrion trivittata*, enough work was done to convince me that the ciliary mechanism of both species operates in the same way.

Finally a careful investigation was made of the relation between muscular and ciliary movements when the snail was fastened to a support with its foot expanded at the surface of the water. Here also the cilia along the anterior border were seen to start beating as muscle contractions occurred, and to decrease their rate of movement as the contractions subsided and stopped. The lateral foot processes were sometimes swung about and this movement was also accompanied by local ciliary activity. When the cilia were beating actively and continuously the anterior border of the foot showed a vibratory motion caused by a series of rapid contractions and expansions. In addition to the more local muscular contractions correlated with ciliary action, there sometimes occurred irregular foot movements of greater distribution which were unassociated with beating cilia. Movements of the latter type were also observed occasionally when the foot was removed from the body.

Conclusions.

The observations and experiments briefly recorded above lead me to believe that locomotion in *Alectrion* is the direct result of ciliary action and not arhythmic muscular movements, and the unusual behavior of the cilia points to the conclusion that they have been brought in one way or another under the control of the nervous system. The fact that a resting snail can be made to move its pedal cilia by stimulating the receptors on one of its tentacles with fish meat is in itself sufficient evidence of the controlling action of the nervous system. The muscular movements, which occur when cilia begin beating and which accompany vigorous ciliary activity, may be explained by assuming that efferent impulses arrive at the ciliated cells and muscles at approximately the same time. It is possible, however, that nervous impulses travel only to the muscles, and that the latter in some way exert a stimulating influence on the overlying ciliated epithelium. Further investigation, both histological and physiological, is planned which, it is hoped, will throw more light on the problem.

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BIOLOGICAL BULLETIN

CLEAVAGE AND MESENCHYME FORMATION IN TOXOPNEUSTES VARIEGATUS.

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This investigation was undertaken for the purpose of determining the origin of the primary mesenchyme in Echinoidea. Although numerous researches have been carried out on the early stages in the development of echinoids, the question of the origin of the mesenchyme has never been definitely settled.

MacBride,[1] writing of *Echinus esculentus*, states that "this mesenchyme originates, almost certainly, from the descendants of the smaller micromeres." The figures which Boveri (1901) gives of the eggs of *Strongylocentrotus lividus* indicate that this is true, and it is believed that unmistakable evidence is herein presented that in *Toxopneustes variegatus* (*Lytechinus variegatus*) the mesenchyme arises from the micromeres.

MATERIAL AND METHODS.

The material on which this investigation was carried out was obtained by Dr. D. H. Tennent, during the summer of 1908, in the course of work at the United States Fisheries Biological Station, Beaufort, N. C.

The segmenting eggs were fixed in either picro-acetic or sublimate-acetic. In both cases the solutions were two per cent. acetic acid. The observations recorded here were made on whole embryos stained with borax carmine and safranin, and on sectioned material stained with borax carmine or iron hæmatoxylin.

In addition to the above material, Dr. Tennent lent camera

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[1] "Text-book of Embryology," p. 507.

lucida sketches of the cleavage stages which he made from living eggs. While it is possible to make out these stages in the preserved specimens, they are much clearer in the living eggs; and his sketches have been used to illustrate the segmentation changes.

EARLY CLEAVAGE.

The *Toxopneustes* egg has been found especially favorable for many kinds of study because of its transparency and beautiful clearness of structure, when fixed as well as while alive.

As this paper deals with a definitely limited phase of development it seems best not to include a detailed description of the egg, or its jelly layer, or of the manner of formation of the fertilization membrane, but to pass at once to the consideration of cleavage.

The first cleavage of the *Toxopneustes* egg is vertical and results in two equal cells being formed about forty minutes after insemination (Figs. 1, 2, 3 and 4). Tennent (1911) gave the characteristic rhythm for *Toxopneustes* cleavage at 28° C., and the time intervals indicated are from his paper. This rhythm may be altered by changes in temperature as has been shown for example by Loeb and Chamberlain (1915) who worked with *Arbacia* eggs. They found a close correlation between the temperature of the water in which the eggs were kept and the period of time elapsing between insemination and the first cleavage, and were, therefore, able to calculate the temperature coefficient for cell division at varying temperatures.

The second cleavage, about one hour and ten minutes after insemination, is also vertical, and divides the egg into four equal cells which are shown in polar view in Fig. 5. After one hour and thirty-five minutes, the third cleavage furrow coming in horizontally divides the egg into eight equal cells (Figs. 6 and 7). This is the last cleavage in which all of the cells are equally divided.

A twelve cell stage is formed by a horizontal division which cuts off four small cells, the first set of micromeres, at the vegetal pole. Practically coincident with this, the four cells at the animal pole are divided by vertical furrows into eight. In the sixteen cell stage there are therefore, three different kinds of cells as regards size, these being respectively, the small macromeres, a group of eight medium-sized cells at the animal pole; the micrometers, four small cells at the vegetal pole; and the large macromeres, four large cells lying between the two groups just mentioned. These are shown as seen from the side in Fig. 8 and as seen from the vegetal pole in Fig. 9. It is one hour and fifty minutes after the addition of the sperm when this stage is complete.

From this time on, all of the cells do not divide simultaneously. Two hours and ten minutes after insemination the four large macromeres are divided vertically into eight, and the eight small macromeres are divided vertically into sixteen. About ten minutes later the micromeres are cut horizontally into eight, but this is an unequal division, the four smaller micromeres lying nearer the vegetal pole (Fig. 10). The completed thirty-two-celled embryo is shown from the side in Fig. 11, and the crowding in of the micromeres can be noticed at this stage.

The next furrow cuts the lower set of eight large macromeres horizontally, and twenty minutes later, or two hours and fifty-five minutes after insemination, vertical cleavages divide the upper set of large macromeres into sixteen, and the sixteen small macromeres into thirty-two. After three hours and five minutes, the upper micromeres are divided vertically into eight, the four micromeres referred to above as lying nearer the vegetal pole, now being crowded back and partially concealed by this ring of eight cells. Five minutes later the lowest set of macromeres is divided by vertical cleavages into sixteen (Fig. 12). Twelve of the seventy-six cells of this stage are micromeres, the later development of which will be taken up presently.

The following table (page 142) shows in a diagrammatic way, the sequence of cleavage in the *Toxopneustes* egg. The time indicated is the elapsed time after insemination.

Theel's (1892) account for *Echinocyamus pusillus* differs from this in that he describes the micromeres as being cut off at the animal pole. According to his description the sixteen-cell stage is completed in about two hours and twenty minutes and is accomplished as follows: "First the four upper segments are divided by a horizontal plane into four small cells or micromeres and

TABLE SHOWING SEQUENCE OF CLEAVAGE.

Time.	40 m.	1 h. 10 m.	1 h. 35 m.	1 h. 50 m.	1 h. 50 m.	2 h. 10 m.	2 h. 20 m.	2 h. 35 m.	2 h. 55 m.	2 h. 55 m.	3 h. 5 m.	3 h. 10 m.
Small macromeres			4	4	8	16	16	16	32	32	32	32
Blastomeres	2	4										
Large macromeres			4	4	4	8	8	16	16	24	24	32
Micromeres				4	4	4	4	4	4	4	8	8
							4	4	4	4	4	4
Total cells	2	4	8	12	16	28	32	40	56	64	68	76

[1] It will be noticed that there are two columns headed 1 h. 50 m. and two headed 2 h. 55 m. This is for the purpose of indicating that the formation of the micromeres is completed before complete division of the four small macromeres, about one hour and fifty minutes after insemination, and that the division of the sixteen small macromeres is completed before that of the upper eight large macromeres, two hours and fifty-five minutes after insemination. The duration of an actual twelve cell and of an actual fifty-six-cell stage is short.

four large ones. Immediately after this segmentation or, though more seldom, before it is accomplished, the four lower segments are divided by a vertical plane into eight almost equal spheres, which become arranged in a more or less irregular manner in two rows."

Further, in *Echinocyamus*, two hours and thirty-five minutes after fecundation, the large macromeres are divided vertically, the small macromeres are cut horizontally, and after fifteen or twenty minutes "the four micromeres at the animal pole become parted by horizontal planes into eight, four of which are smaller, contain a clearer protoplasm and, as they lie in the same plane, constitute the animal pole itself; the four remaining ones have their place somewhat outside and below." It will be noticed that the small macromeres are divided horizontally in *Echinocyamus*, while in *Toxopneustes* the division is vertical.

Theel fell into error concerning the micromeres probably because he assumed that they were homologous with the micromeres of flatworms, annelids and molluscs. Boveri ('01) in his study of the egg of *Strongylocentrotus lividus*, demonstrated that it is at the vegetal pole that the micromeres are separated at the twelve-cell stage.

FORMATION OF MESENCHYME.

Very early in the development of the *Toxopneustes* blastula the cells which are to give rise to the future mesenchyme can be distinguished. With the cutting off of the micromeres there is separated from the rest of the embryo that protoplasm which later goes to form the mesenchyme. These cells, or cells derived from them, must pass from the wall of the blastula into its cavity; and as early as the sixteen-celled stage some of the first four micromeres exhibit this tendency to migrate inward. Sections passing through the poles of a sixteen-celled stage show the micromeres visibly crowded. Figs. 13 and 14 are sections of two different individuals in this stage, and the micromeres at the vegetal pole are projecting slightly into the blastocœle with their inner ends bulging as a result of pressure from the adjacent cells in the wall. The difference in the number of the micromeres in the two figures is due to the planes of the sections. Fig. 13 is cut parallel to the short axis of the group of micromeres, while Fig. 14 is cut parallel to the long axis of the group.

A later stage is shown in Fig. 15 which is a somewhat oblique section through the micromeres. In this case the micromeres have multiplied and the process of pushing in has continued until one cell of the particular section in question has lost all connection with the surface.

Figs. 16*a*, 16*b* and 16*c* represent three serial sections of one specimen which is slightly older than the preceding one. Here the migration is quite evident. From this time on the micromeres are not readily distinguishable by their small size as heretofore, since by an unequal rate of division, all the cells have become more nearly the same size. The fact that in earlier stages it was the micromeres, and they alone, which passed inward, and the fact that throughout the stages the migration is restricted to a limited area in the wall, afford proof that in the later stages the mass of cells which has passed inward represents the offspring of the original micromeres.

A more pronounced wandering in of cells is shown in Figs. 17*a*, 17*b* and 17*c*—serial sections of one embryo. Here, certainly, pressure from without has ceased to operate as the cause of migration, for the wandering cells are free to a great degree from contact with cells of the wall.

There have been various theories of the cause of the separation of the mesenchyme cells from the wall of the blastula in other forms. Grave, in *Ophiura brevispina*, describes the mesenchyme cells as being pushed or squeezed inward by the coercive pressure of the organism as a whole, though this is not the whole story of the process. MacBride describes the migration of mesenchyme cells in *Asterias vulgaris* thus: "The 'wandering' of these cells seems to be effected by their emitting long filamentous pseudopodia which span the blastocœle, and along these strands the body of the cell glides like a drop of dew on a spider's web."

Serial sections through an embryo figured in 18*a*, 18*b* and 18*c*, show the mesenchyme cells much more numerous, and those most recently budded off are compactly pressed together. In Fig. 19 the cells have multiplied still more and the mesenchyme lies close to that part of the wall from which it arose. Fig. 20, a stage still later, shows the mass of mesenchyme cells much larger but still compact. In this set of material this condition was found one hour and forty minutes before the first indication of gastrulation, namely, the flattening of the vegetal pole.

When the process of gastrulation begins, the mesenchyme cells migrate laterally and become arranged in a ring in the blastocœle about the polar axis of the embryo. The invagination of the archenteron proceeds from the vegetal pole toward the space unoccupied by mesenchyme. Fig. 21 is a very early gastrula with the mesenchyme cells lateral to the invaginating archenteron. A horizontal section through an early gastrula in approximately the same stage as Fig. 21 is shown in Fig. 22. The ring of mesenchyme is distinctly visible around the archenteron. A later gastrula is shown in Fig. 23 in which the secondary mesenchyme is being proliferated from the inner end of the archenteron. The primary mesenchyme may be seen at the sides of the archenteron. This specimen was somewhat distorted in sectioning and for comparison, Fig. 24, drawn from a camera lucida sketch of the living gastrula, has been inserted.

Since the publication of Theel's paper, in which the literature on the subject up to 1892 was discussed, the origin of the mesenchyme has been described for several echinoderms. Like the earlier accounts, these, with the exception of those of Boveri and MacBride, deal rather with the general question as to whether the mesenchyme arises in the blastula at the vegetal pole, or in the gastrula from the archenteron, either during or after its invagination, than with the idea of associating the primary mesenchyme with an early differentiation in the segmenting egg.

The following table gives a resumé of the more recent literature, with the inclusion of Bury's observations of earlier date on *Antedon*.

TABLE SHOWING TIME OF MESENCHYME FORMATION.

Date.	Author.	Form.	Time of Mesenchyme Formation.
		ASTEROIDEA.	
1892	Field.	*Asterias vulgaris.*	Blastula, through gastrulation.
1896	MacBride.	*Asterina gibbosa.*	After complete gastrulation.
1902	Masterman.	*Cribrella oculata.*	After complete gastrulation.
1912	Gemmill.	*Solaster endeca.*	As morula changes to blastula.
1914	Gemmill.	*Asterias rubens.*	Late gastrula.
		OPHIUROIDEA.	
1897	MacBride.	*Ophiothrix fragilis.*	Blastula.
1916	Grave.	*Ophiura brevispina.*	Blastula.
		ECHINOIDEA.	
1901	Boveri.	*Strongylocentrotus lividus.*	Blastula.
1903	MacBride.	*Echinus esculentus.*	Blastula.
		" *miliaris.*	Blastula.
		" *acutus.*	Blastula.
1904	Schmidt.	*Echinus microtuberculatus.*	Blastula.
1914	MacBride.	*Echinocardium cordatum.*	Blastula.
1914	Tennent.	*Cidaris tribuloides.*	Gastrula.
		CRINOIDEA AND HOLOTHUROIDEA.	
1898	Clark.	*Synapta vivipara.*	Gastrula.
1909	Edwards.	*Holothuria floridana*	Blastula at beginning gastrulation.
1888	Bury.	*Antedon rosacea.*	Gastrula.
1892	Seeliger.	" "	Gastrula.

It will be seen that among the Asteroidea there are some differences between various forms in their methods of mesenchyme formation. Field (1892) described these cells in *Asterias vulgaris* as being given off from the developing archenteron even from the beginning of its invagination. Gemmill (1914) found no mesenchyme cells in the normal larvæ of *Asterias rubens* until gastrulation was well advanced, when they appeared at the end of the archenteron. MacBride (1896) found in *Asterina gibbosa* that no mesenchyme was formed until after gastrulation was complete. In connection with these accounts it is interesting to note the results of Herbst (1896) who experimentally induced larvæ of *Asterias glacialis*, which normally produce mesenchyme only after gastrulation, to form large numbers of mesenchyme cells while still in the blastula stage.

In contrast with these processes, Masterman (1902) in *Cribrella oculata*, and Gemmill (1912) in *Solaster endeca*, have described a very different series of events. In *Solaster* the cleavage results in a solid morula of nearly equal cells. The outside of this mass then becomes wrinkled by the appearance of convoluted furrows which so increase the surface that the cells inside can become arranged in a single layer, leaving a flattened, irregular cavity, the blastocœle. The furrows gradually disappear as the cells over the entire surface become longer and narrower until the larva becomes smooth once more. The initiation of the process of gastrulation also aids in flattening the surface. During these changes some wandering cells fail to arrange themselves in a layer with the others and are left behind in the cavity of the blastula. These are the primary mesenchyme cells. In *Cribrella* similar phenomena occur, but instead of being left behind as the morula changes to a blastula, the mesenchyme cells are budded off after invagination, from the "hypoblast" into the blastocœle, in the manner of the more typical Asteroidea. In addition to this, cells morphologically similar to mesenchyme cells are budded off from the other side of the "hypoblast" into the archenteron, and were called "hypenchyme" by Masterman.

Even fewer species of the Ophiuroidea have been investigated on this point. Grave (1916) in *Ophiura brevispina* found a large number of mesenchyme cells in the blastocœle of early stages. MacBride (1897) observed mesenchyme in blastulæ of *Ophiothrix fragilis* between four and five hours after fertilization, while gastrulation did not begin until about the eighteenth hour.

The majority of Echinoidea show early mesenchyme formation. MacBride (1903) figured blastulæ of *Echinus miliaris*, *E. acutus* and *E. esculentus* with mesenchyme forming at one end, which was, in some cases, slightly flattened. In *E. esculentus* he found about fifty mesenchyme cells which were probably derived from the micromeres in the blastula. In *Echinocardium cordatum* (MacBride 1914) mesenchyme is found at the vegetal pole long before any signs of gastrulation. MacBride was unable to confirm the observations of Fleischman (1888) on this form with regard to the four arch-mesenchyme cells which Fleischman found. Schmidt (1904) observed mesenchyme cells in the blastula of *Echinus microtuberculatus*. They were present at the seventeen-hour stage and continued to be formed until the twenty-four hour stage. Boveri (1901) in his work on *Strongylocentrotus lividus*, found in the unsegmented egg a portion at the vegetal pole that was unpigmented. This region gave rise to the micromeres, and they in turn to mesenchyme in the blastula stage. The lack of pigment in these cells made their development easy to follow.

Tennent (1911) noted the presence of four mesenchyme cells in the blastocœle of the *Toxopneustes* blastula about eight hours after the fertilization of the egg and about an hour before the beginning of gastrulation. An exception to the typical echinoid condition is shown by *Cidaris tribuloides* (Tennent, 1914), in which the formation of the mesenchyme is delayed and is given off from the inner end of the archenteron in the gastrula.

To our knowledge of mesenchyme formation in the Holothuroidea the observations of Clark (1898) that in the blastula of *Synapta* there is no evidence of mesenchyme cells, but that they arise later, wandering in from the whole surface of the archenteron, and of Edwards (1909) that in *Holothuria floridana* the mesenchyme begins by cell-proliferation at the vegetative pole of the blastula, while at the same time gastrulation takes place, confirm the earlier evidence of Korschelt, Selenka and Ludwig to the effect that in the Holothuroidea the time of mesenchyme formation varies.

Bury (1888) and Seeliger (1892) from their study of the development of *Antedon rosacea* agree that the formation of the mesenchyme does not begin until after the beginning of the processes of gastrulation.

From these accounts it may be seen that, with the exception of Boveri's work on *Strongylocentrotus* and MacBride's work on *Echinus esculentus*, little attempt has been made to trace the origin of the mesenchyme cells further back than to the approximate point in the larva from which they arise. If formed early they come from the vegetal pole of the blastula, if late from the archenteron. Theel makes the statement that "the mesenchyme arises earliest, with very few exceptions, in those groups of echinoderms in which the larvæ have their skeleton earliest developed." Since one of the functions of the primary mesenchyme is the formation of the skeletal rods of the larvæ the reason for this correlation is evident.

In this paper it has been shown that the primary mesenchyme in *Toxopneustes* comes from the micromeres. Driesch (1900) has shown by experiments on fragments of blastulæ, that after the micromeres have been formed, the cells at the opposite pole of the larva are unable to form mesenchyme. The micromeres arise at the vegetal pole of the segmenting egg, but cannot be considered as either ectoderm or endoderm. The primary mesenchyme is the result of an earlier differentiation than either ectoderm or endoderm.

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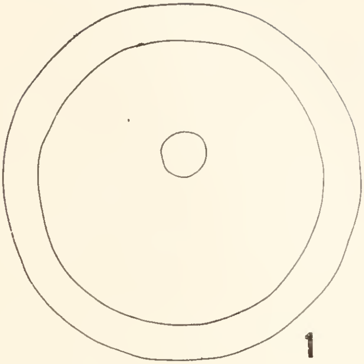
DESCRIPTION OF PLATES.

Figs. 1 to 12 and Fig. 24 were made from camera lucida sketches of living eggs. A pantograph was used to enlarge them two and one half times, and this gave a magnification of 1,050 diameters. They have been reduced one half in reproduction so that the magnification is now 525 diameters.

Figs. 13 to 23 were made from camera lucida drawings of mounted sections which were enlarged two and one half times and reduced one half giving a final magnification of 725 diameters.

Explanation of Plate I.

Fig. 1. Fertilized egg.
Fig. 2. Incomplete 2-celled stage.
Figs. 3 and 4. 2-celled stage.
Fig. 5. Polar view of 4-celled stage.
Fig. 6. Incomplete 8-celled stage.



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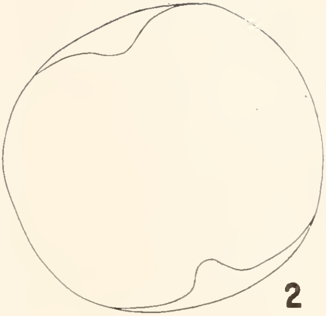

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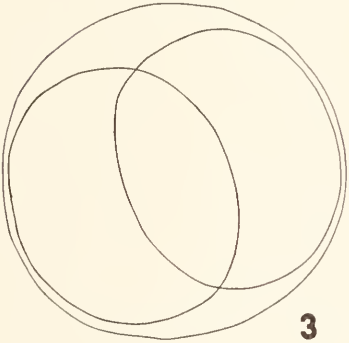

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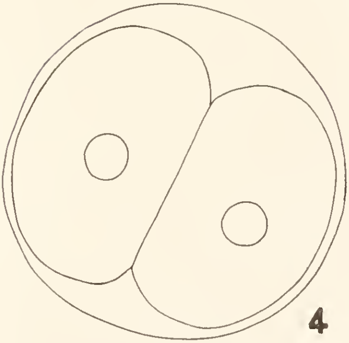

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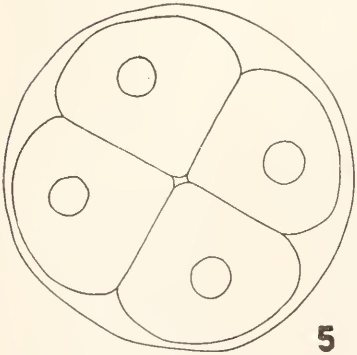

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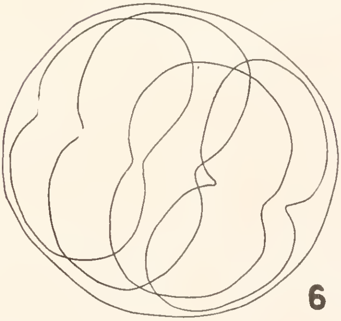

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GUTHRIE AND HIBBARD.

Explanation of Plate II.

Fig. 7. Side view of 8-celled stage.

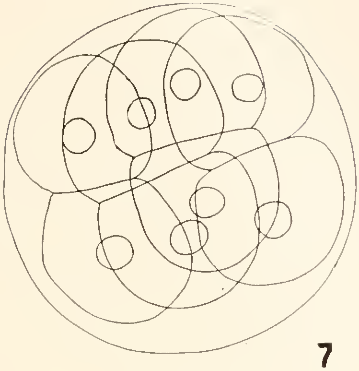
Fig. 8. Side view of 16-celled stage showing the micromeres cut off at the vegetal pole.

Fig. 9. View of 16-celled stage from vegetal pole.

Fig. 10. View of 32-celled stage from vegetal pole showing second set of micromeres.

Fig. 11. Side view of 32-celled stage.

Fig. 12. Polar view of a 76-celled stage showing the twelve micromeres.



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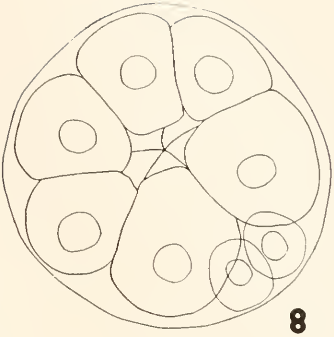

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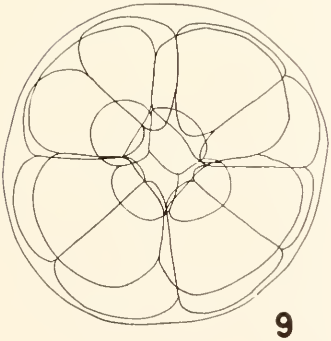

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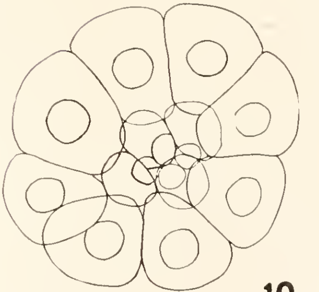

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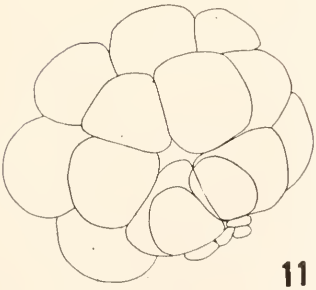

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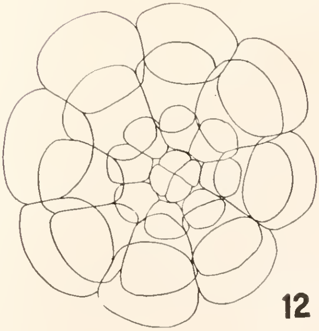

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GUTHRIE AND HIBBARD.

Explanation of Plate III.

Fig. 13. Section through a 16-celled stage passing through the short axis of the group of micromeres.

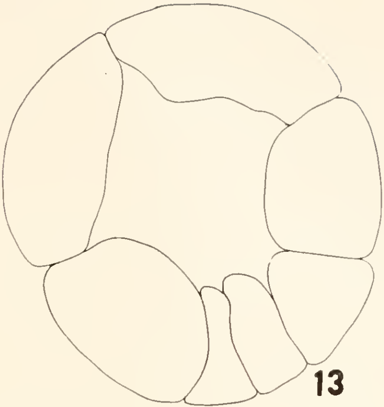
Fig. 14. Section through a 16-celled stage passing through the long axis of the group of micromeres.

Fig. 15. Section of slightly older stage showing multiplication of micromeres.

Figs. 16*a*, 16*b*, 16*c*. Serial sections of an older embryo showing migration of micromeres.

Figs. 17*a*, 17*b*, 17*c*. Serial sections of a still older embryo showing further migration of micromeres.

Figs. 18*a*, 18*b*. Two of a series of sections showing more numerous mesenchyme cells (micromeres).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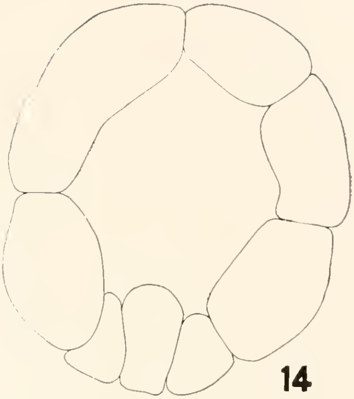
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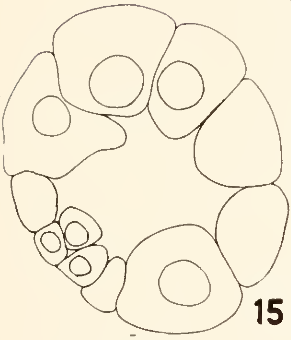
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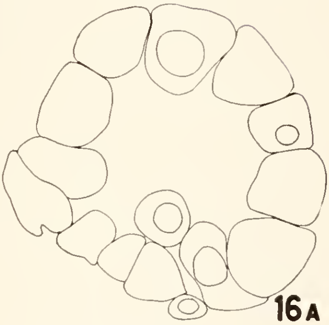
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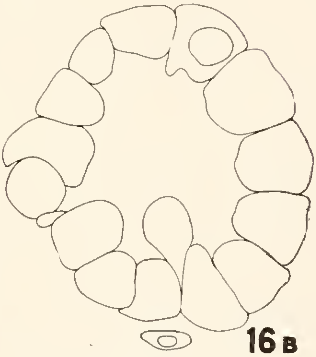
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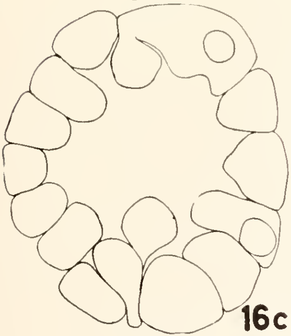
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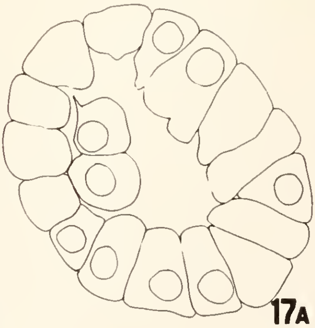
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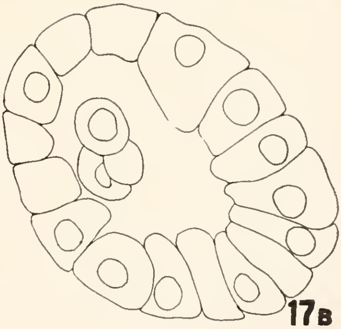
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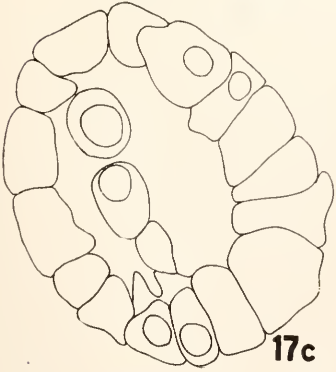
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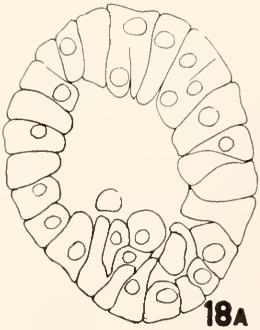
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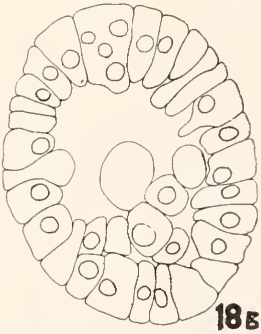

18B

GUTHRIE AND HIBBARD.

EXPLANATION OF PLATE IV.

FIG. 18*c*. Third section in the same series as Figs. 18*a* and 18*b* showing more numerous and compact mesenchyme cells.

FIG. 19. Section through slightly older embryo showing continued multiplication of mesenchyme cells.

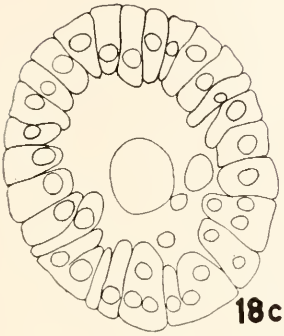
FIG. 20. Section through a later stage than Fig. 19.

FIG. 21. Vertical section through an early gastrula.

FIG. 22. Horizontal section of same stage as Fig. 21.

FIG. 23. Vertical section of older gastrula.

FIG. 24. Entire living gastrula.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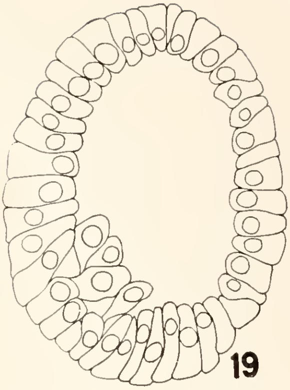
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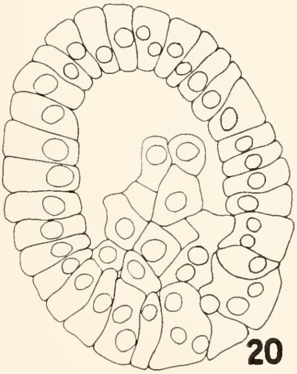
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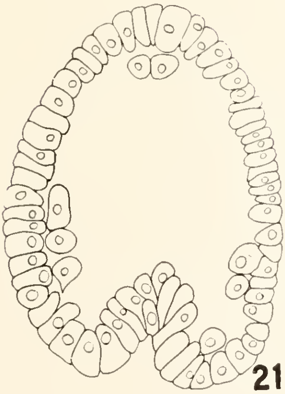
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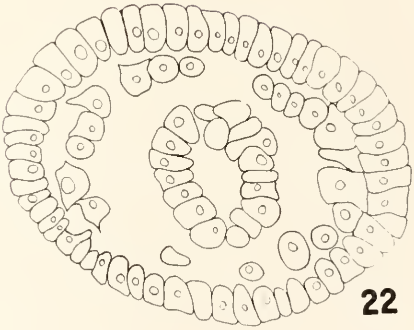
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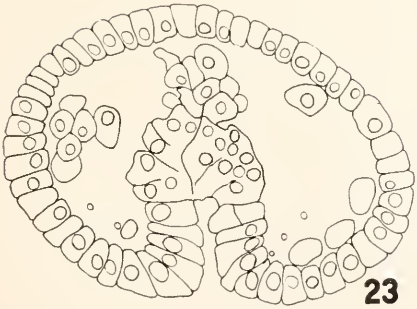
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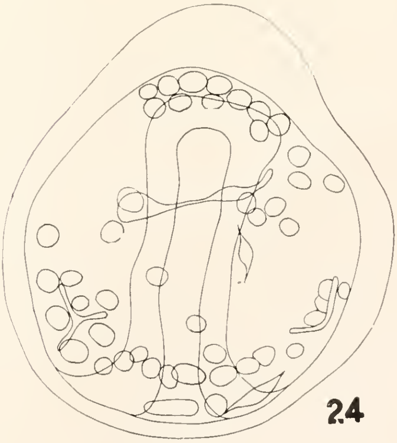
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THE EFFECT OF ADRENIN UPON THE RATE OF LOCOMOTION OF PLANARIA AND OF TOAD LARVÆ.

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Biological Laboratory of Bryn Mawr College.

In his investigation of the chromaffine system of annelids, Gaskell (2) has pointed out the close morphological and physiological relationship which exists between the chromaffine, the sympathetic and the contractile vascular systems, wherever these are found. He holds that the presence of any systems corresponding physiologically to the first two, necessarily implies the presence of the third on which to act; and in support of this view, he finds in the annelid group, in those forms which possess a contractile vascular system, a cell which he considers the common ancestor of the sympathetic and chromaffine systems. If, therefore, the earliest indication of chromaffine tissue is found among the annelida, it may be safely assumed that in the platyhelminth group in which there is no contractile vascular system, there is nothing comparable to the adrenin-producing cells of higher forms. The effect of adrenal extract is not, however, confined to the regulation of the cardio-vascular apparatus, although this seems to be one of its chief functions. Elliott (1) has definitely shown that it affects other forms of smooth muscle in a manner similar to the sympathetic nervous system, causing either an excitation or an inhibition, according to the nature of the sympathetic fibers supplying the tissue. Its influence is, furthermore, not even limited to smooth muscle for skeletal muscle is affected, also, as various investigators have demonstrated. Is it possible, then, that this secretion of the adrenal medulla exercises an influence upon the contracility of the simple muscles of an organism such as *Planaria*, which does not itself manufacture adrenin?

Bands of circular, longitudinal and diagonal muscles are found in the body wall of planarians; and it is by means of these that locomotion is made possible. It had been generally believed that only the crawling movement is accomplished through muscular contractions, and that gliding results chiefly from the beating of ventral cilia; but Stringer (8) has shown that when muscular activity is entirely checked locomotion does not occur, even though the cilia are beating vigorously; and she concludes that the "locomotion of planarians is essentially a muscular act in which the cilia play no necessary part."

Although the influence of adrenin upon the muscular contractility of *Planaria* cannot be exactly homologized with that upon vertebrate muscle, it is well to bear in mind the results of these investigations. Oliver, and Schäfer (7) report both a greater and a more prolonged contraction of the gastrocnemius in frogs as the result of adrenin injection, and also an increase in muscular activity following administration of the extract by mouth. Takajasu (9), however, working upon isolated muscle, obtained just the opposite effect. He found that the curve of contraction in skeletal muscle, immersed in solutions of adrenin, varying from 1 : 500,000 to 1 : 5,000, becomes successively lower and of shorter duration, although there occurs a preliminary period during which the contractions are slightly prolonged. Gruber (3 and 4) has obtained interesting results with muscle preparations in which fatigue has been induced either electrically or chemically. In these cases, adrenin, instead of causing a still further lowering of the fatigue curve, really produces a rapid recovery of the normal irritability of the muscle. Unlike Oliver and Schäfer, however, he reports no improvement in muscles not fatigued. These investigators, it is evident, have employed various means of introducing the extract, and have worked upon both entire animals and isolated muscle. With planarians, injection is impracticable, and the immersion of isolated muscle altogether impossible; but by placing entire individuals in solutions of adrenin, a combination of the methods of immersion and feeding is obtained.

The experiments here described were carried out upon *Planaria doratocephala*, and *Planaria velata*. The worms were placed in large finger bowls, containing spring water, and kept in the laboratory at as nearly constant temperature as possible. Food consisting of finely minced earthworms of raw meat, was supplied two or three times a week, and at each feeding the water was changed. The Parke, Davis, and Company preparation of adrenalin chloride[1] was at first used; but it was found unsatisfactory, as animals immersed in solutions varying from 1 : 1,000 to 1 : 20,000 soon became inactive, and died within less than an hour. Solutions of the same strength, however, prepared from adrenin in tablet or in crystal form did not produce the same effect; and it seemed not improbable that it was the chloretone in the first preparation which caused the rapid loss of activity. To test this hypothesis, a solution of chloretone was prepared, equal in strength to that contained in the adrenalin chloride preparation, and a comparison was made of the effects upon *Planaria* of similar dilutions of these two. In both cases complete loss of locomotor power quickly resulted. The chloretone alone (1 : 2,000) was sufficient to cause within an hour, a noticeable elongation and lack of response to mechanical stimuli, an effect which is not surprising, since chloretone of 1 : 1,000 strength is recommended as a ready anæsthetic for planarians (10).

In the following experiments, therefore, adrenin in tablet and in crystal form was used. In order to determine whether or not this extract exercises any influence whatever upon *Planaria*, active individuals were put into small bottles containing solutions of adrenin of different strengths, and subjected to the influence of the extract for varying periods of time. It was necessary to keep the bottles corked to prevent the rapid oxidation of the adrenin, but there was enough oxygen present to sustain the worms; and the fact that planarians in corked bottles containing water only were not affected seems proof that the results noted cannot be attributed to lack of oxygen. In the preliminary experiments it was found that animals in solutions of adrenin, varying from 1 : 1,000 to 1 : 15,000 died in less than three days, but in more dilute solutions they remained alive and active even after a week. Most of the later experiments were, therefore, made with a 1 : 15,000 solution. for one day, or with stronger solutions for a shorter period.

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[1] Each fluid ounce of this preparation contains $\frac{2}{5}$ grain of adrenalin chloride and $2\frac{1}{4}$ grains of chloretone.

It was difficult to find a means of measuring the influence of the extract upon the musculature. After unsuccessful attempts to record the effect through electrical stimulation, a method of testing muscular activity by means of the rate of locomotion was adopted. A narrow trough, 6.5 cm. long by .3 cm. in width, was made by cementing two ordinary glass slides, side by side, upon a glass plate. Planarians, put into the trough with a little water, readily progressed from one end to the other, usually in the angle formed by the bottom plate and the edge of one slide. A starting place was then marked off; and with a stop-watch the rate of travel was measured. Only continuous non-stop journeys, from the time the head passed the starting point until it reached the end of the trough, were recorded. A large variation was found both in successive rates of the same individual, and in rates of different normal individuals; but by taking the average of a number of successive rates for each animal, and by using the same ones both before and after treatment with adrenin, it was possible to secure results which seem to be significant.

The animals were handled as far as possible in the same way in each experiment; and to avoid differences due to the influence of light, the trough was placed on the table in such a position that the animals moved away from the window in a direction parallel with the entering rays of light. Parker and Burnett (6) have shown that planarians have a "tendency to turn away from the course when directed toward the source of light," and this was easily verified, as it was found almost impossible to keep a worm traveling toward the window. In eighteen successive trials only once did an individual finish the whole length toward the light without reversing; but in the other direction full trips were readily obtained.

In testing the effect upon locomotion, the normal rates of the planarians in water were first recorded, then the rates immediately after exposure to the influence of adrenin, and finally the rates after recovery in water. In the first series (Table I.) ten individuals were used. Only two rates for each were taken under each of the three conditions; but a comparison of the averages of the twenty rates thus obtained indicates an effective influence of adrenin upon the locomotor activity. The average normal rate was 53.4 seconds; but after one day in adrenin solution (1 : 15,000) the average time needed to travel the same distance was increased to 69.5 seconds. Furthermore, as an indication that the change was in response to a definite stimulation, and not merely the expression of a generally weakened physiological condition, such as does occur after a time when the animals are kept in the laboratory, there was a decided improvement after the animals had been placed in water again for two days. The rate this time was even better than the original, 51 seconds.

This same retarding influence of adrenin, followed by recovery in water was found in the next five planarians tested. But the third Series, as is shown in Table I., gave apparently very disappointing results. It may be interesting, however, to note that the animals of Groups B and C which gave good initial rates had been tested before with adrenin. The very poor rates, therefore, of the planarians of Groups A and D, which had been kept for some time in the laboratory, and had not been previously used, indicate that these animals were no longer in the same condition as those of the first experiments and might well be affected differently by the same extract.

Fresh planarians were then obtained and the same tests repeated. The results of these, also recorded in Table I., seem to confirm the conclusion drawn from the first experiments, that adrenin produces a decrease in the normal rate of locomotion of *Planaria*, which is overcome when the animals are again placed in water.

The nature of the effect of adrenin upon the muscular contraction of *Planaria* is not, however, as simple as the decrease in the rate of locomotion would indicate. Observation of the animals reveals clearly that the effect is not merely one of weakened muscular response to mechanical and contact stimuli. There seems to be no delay in the time of reaction to stimulation, and no constant decrease in the strength of the contraction. It is moreover, apparently not the same effect as that which Moore (5) found produced by strychnine, as no reversal of the normal response to stimulation was observed at any time after treatment with adrenin. Experimental animals, touched with a camel's hair brush, reacted in the usual manner by contracting the longitudinal and relaxing the circular and transverse muscles. The effect was rather one of excitement, confined chiefly to the anterior end of the body, which seemed to make coördinated locomotion difficult. The animals expended their energy in excited movements of the head, and occasionally were unable even to proceed the whole length of the track. When continuous trips were obtained, a decrease in rate was found, as has been noted; but this cannot be entirely due to a decrease in muscular activity, as there is frequently clear evidence of an increased irritability. At other times, however, the excitatory effect was not noted, and the slower rate was really indicative of a slower rhythmic contraction as the animal progressed along the trough.

Table I.

Series.	Strength of Sol.	Number of Animals.	Time Exposed to Sol.	No. of Rates Taken.	Average Initial Rate in Sec.	Average Rate in Adrenin.	Average Rate After Return to Water, 1–3 Days.
1.	1 : 15,000	10	24 hrs.	2	53.4	69.5	51
2.	1 : 15,000	5	19 hrs.	2	about 55.	84	59
3. A	1 : 15,000	4	24 hrs.	2	126.5	86.6	66.6
B	1 : 15,000	3	24 "	2	59.2	73.8	64.2
C	1 : 15,000	3	24 "	2	51.5	47.5	60.8
D	1 : 15,000	2	24 "	2	187.	85.5	103.5
E	1 : 15,000	3	22 "	5	69.	67.3	——
4. A	1 : 2,000	1	15 min.	5	91.8	139.8	131
B	1 : 2,000	1	20 "	5	71.6	206	137.5
C	1 : 20,000	3	30 "	5	41.1	48.2	37.2
D	1 : 10,000	4	20 "	5	33.1	52.2	44.3
E	1 : 10,000	4	20 "	5	33.1	51.2	41
F	1 : 10,000	2	20 "	5	34.8	50.9	30.4
G	1 : 10,000	2	20 "	5	30.6	38.7	25.7

This variability of result, expressed as it is in both cases by a decrease in rate, is difficult to explain; and it may be that an explanation is superfluous, and adrenin is not specific in its influence upon planarian muscle. It is possible, however, that we are dealing here with a definite action of the extract; and that a more detailed study of the effect upon the three kinds of muscles, and a more accurate determination of the physiological condition of the individuals at the time they are tested may throw more light on the subject. A possible explanation may be found in the duration of the influence of the extract. The animals which showed to a greater extent the symptoms of excitement were those which had been treated with adrenin for an hour or less. Those which were in the solution for a day, with the exception of some of the groups of Table I., Series 3, showed almost uniformly a real slowing of the rate of muscular contraction. It may be that the initial effect produced by adrenin upon the muscular apparatus of planarians is excitatory in nature, and that the increased irritability, expressed in useless and non-coördinated movements really results in a decrease in the rate of locomotion. As the animals are left in the solution, however, the exciting effect wears off, and there results either an approximate return to normal, as in Series 3, Groups C and E, or a decrease in irritability, following naturally the undue excitement created at first.

This hypothesis of an initial excitatory effect might account also, for some of the other discrepancies of Series 3. The animals of Groups A and D, which showed such a poor initial rate in water may have been in a condition of lowered vitality, perhaps a state of fatigue. Here, as the results show, there followed an increased rate after treatment with adrenin. Might it not be possible that the excitatory effect of the extract was, in these cases, merely sufficient to raise the muscular tone to normal, without producing any symptoms of over-excitation? It is not unlikely that in these animals the increase in the rate of locomotion indicates the real effect of adrenin, which in most of the other experiments is marked by the decrease in rate due to the useless movements of excitement. This possibility may be lessened, perhaps, by the fact that the animals of Group A continued to improve even more after return to water, while those of Group D fell back, indicating that other factors were at work; but this does not exclude the view that adrenin produces a temporary improvement which may or may not become more lasting, dependent upon these other factors.

In order to determine whether or not the same results are produced by repeated doses of adrenin, several further tests were made, the results of which are recorded in Table II. After each treatment with the extract, one to three days in water were allowed for recovery before the next tests were made.

Such variable results were obtained, however, that it is impossible to say whether there is a tendency for *Planaria* to become less sensitive to repeated doses or not. By looking over the percentages of decrease it may be seen that eleven of the twelve animals responded to a lesser degree to the second treatment than to the first; but there is one great exception, and there is, in addition, the result, not recorded in the table, of a third test made with the last series where the decrease was 47 per cent., less than the first but greater than the second. Such variation was to be expected, from the nature of the problem, since the difference in the rate of locomotion does not express the whole of the effect. Although these last experiments add nothing new to our knowledge of planarian reaction to adrenin, they do distinctly confirm the fact that adrenin produces a slowing of the rate of locomotion through an over-excitability which expends itself to no effect.

Table II.

Series.	Strength of Sol.	No. Animals Used.	Time Exposed to Sol., Min.	No. Rates Taken.	Ave. Initial Rate in Sec.	First Adrenin, Rate.	Per Cent. Decrease.	Ave. Rate After Return to Water, 1–3 Days.	Second Adrenin, Rate.	Per Cent. Decrease.	Ave. Rate After Return to Water, 1–3 Days.
1	1:10,000	4	20–30	5	40	56.15	40.3	34.8	45.4	30.4	33.2
2	1:10,000	3	25–45	5	29.46	47.35	60.7	33.8	43.8	29.6	38.4
3	1:10,000	1	20	5	45.2	43.7	−03.3	36.2	60	65	53
4	1:10,000	4	20	3	38.4	64.7	68	45.3	57.9	27	41.9

The innervation of the muscles of *Planaria* has not yet been worked out, but it is probable, from the simple character of the nervous system, that the action of adrenin is directly upon the muscle and not through the nerve endings. This point, however, could not be successfully tested. In an attempt to ascertain the rôle of the nervous system it seemed desirable to compare the reactions of the anterior ends of the animals with those of the brainless parts, when exposed to the same solutions of adrenin. A number of individuals were cut in two just posterior to the brain region, and the wounds allowed to close over. It was practically impossible, however, to obtain any satisfactory rates from these pieces even in water, as the small head ends could not be kept in a straight path, and the larger posterior parts moved too slowly in the beginning for any effect of adrenin to be noticeable. It may be noted in this connection that Gruber (3) obtained the same results with denervated vertebrate muscle as with normal nerve-muscle preparations.

Before concluding it may be of interest to mention the results of a few experiments upon toad larvæ. Only fifteen tadpoles were used; and although the results obtained are by no means conclusive they indicate several possibilities which may lead to further investigation. Tadpoles of two ages were used, measuring during the period of experimentation from 8 to 10, and 14 to 16 mm. in length. The rates of locomotion were determined by the time needed to swim around a circular track .7 cm. wide and 25 cm. in circumference. This method was suggested by the habit frequently observed in normal tadpoles of swimming around the sides of the bowls in which they are kept.

Very young tadpoles seemed unaffected by a solution of adrenin crystals 1 : 10,000, although older ones showed some response. Solutions of 1 : 1,000 strength were therefore used, and a slight decrease in rate was obtained. As with *Planaria*, this slowing was again due to a greatly increased excitability which made sustained action impossible. After exposure to the extract the tadpoles reacted spasmodically to mechanical stimulation, swimming about in a highly excited state for a short time; but they soon become exhausted and unable to react until after a period of rest. This state of excitability followed by exhaustion was more pronounced in the larger than in the smaller tadpoles, probably indicating an increased sensitivity.

Adrenalin chloride of the same strength had a very powerful effect, producing absolute paralysis of movement in less than a minute. If the animals were removed at once, however, and allowed to recover in water for an hour, the same excitability and lack of endurance was noted as with the solution of adrenin crystals. This seems to bear out the suggestion made at the beginning that it is the chloretone in the preparation which causes the immediate stupefying effect. The same paralysis, moreover, is produced by a solution of chloretone of strength equal to that contained in the adrenalin chloride solution.

With a less concentrated solution of adrenalin chloride, 1 : 10,000, the same effect was produced. At the end of an hour the animals appeared dead; but after another hour in water, the paralyzing effect wore off and they became very active, but unable to keep up any sustained swimming movements. Kept in water for several days, however, the animals, with one exception, completely recovered, and traveled once more at their normal rate. Adrenin, therefore, seems to have an effect upon larval vertebrate muscle as well as upon the simpler muscle of planarians.

This work was carried out under the direction of Dr. Florence Peebles, to whom I wish now to express my sincere appreciation.

SUMMARY.

1. There is a decrease in the rate of locomotion of planarians after treatment with solutions of adrenin, followed by a return to the normal rate when the animals are again placed in water.

2. In those individuals which have not been subjected to the influence of the extract more than an hour, this decrease in the rate of locomotion seems to be due rather to an increased excitability and lack of coördinated movement than to a slowing of the rate of muscular contraction.

3. Planarians exposed to the action of adrenin for a longer period do not give evidence of extreme excitability, but seem to show a real decline in activity, which may be a secondary effect following the initial excitation.

4. Those planarians whose initial rate indicates a state of depression travel more rapidly after treatment with adrenin. This would seem to confirm the suggestion that the effect of this extract upon planarian muscle is predominantly excitatory.

5. Although no definite statement can be made concerning the effects of adrenal extract upon the muscular contraction of toad larvæ, the indications are that they are similar to the effects produced upon the muscular activity of planarians, and that this effect increases with the age of the individuals.

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EYE FACET NUMBER AS INFLUENCED BY TEMPERATURE IN THE BAR-EYED MUTANT OF DROSOPHILA MELANOGASTER (AMPELOPHILA).[1]

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The effect of temperature upon facet number in the red bar-eyed *Drosophila* was studied in connection with an extended series of experiments on the effect of selection which is in progress in the Zoölogical Laboratory at the University of Illinois.

For the higher temperatures (27°–30°) a bacteriological incubator, varying not more than one degree centigrade was used. The room temperature controlled by a Johnson regulator, recorded by means of a thermograph, and varying not more than 2°, was used for medium temperatures. A cold plate arranged by allowing tap water to flow around a dish in which the *Drosophila* bottles were placed and varying not more than 1° during any one experiment, was used for the low temperature.

The parents in each experiment were taken from mass cultures or stocks, no attempt being made to obtain virgin females for this work. However, the average facet number of the parents of any two sets in an experiment differed but little, any difference occurring being much less than the difference between the averages of the offspring raised at different temperatures. In experiments 14, 15, 17, and 18 the parents were left in the bottles for one day only; in all other experiments they were allowed to remain for nearly the full developmental period in order that a larger number of individuals might be obtained.

That light is not an important factor for facet number was determined by control experiments.

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[1] Contribution from the Zoölogical Laboratory of the University of Illinois, no. 141.

The manuscript of Mr. Seyster's paper essentially in its present form was completed in May, 1917, when he entered the U. S. Army Training Camp. At that time it was hoped that he might eventually follow up the work but those plans have not materialized and it seems best to publish the results as they stand. C. Z.

In counting facets, a no. 4 eye-piece and a no. 3 Leitz objective were used. In some cases the facets were counted after the flies had been preserved in alcohol, but mostly the counts were made shortly after the emergence of the flies from the pupa cases. In order to determine the per cent. of error made in counting, a set of ten flies, five males and five females was counted at two different times. The total number of facets in the first count was 1,347, in the second count 1,356, and the per cent. of difference between the two counts was 0.81 per cent. For a larger number of individuals the per cent. of error would probably be less than this; for single individuals it was found to average about 5 per cent. to 7 per cent.

The area of the eye in arbitrary units was found by making an outline camera lucida drawing of the eye, and measuring the enclosed area with a polar planimeter, which was found to give accurate results to 2/100 of a square inch.

DATA.

Experiments 1, 2, 3 (Tables I. and II.).—These experiments were designed originally to test the effect of different amounts of food on facet number. Three bottles, each containing a different amount of food and thirteen pairs of flies as parents, were placed in a small well-lighted room where the temperature was kept at approximately 27°. After seven days the parents were taken out and their facets counted. It was found that the three bottles differed slightly from one another in facet number but that their

TABLE I.

EXPERIMENTS 1, 2, 3, MALES.

	Parents.				Offspring.				Difference Between Parents and Offspring.	Stock at End of Experiment.
Temperature...	(21°–23°)				27°					19–21
Catalog number....	1	2	3	Total	1	2	3	Total		
Number of individuals...........	11	10	8	29	25	20	21	66		24
Facet averages......	105	112	106	108	85	86	77	82	26	164
Probable errors....				±3.0				±1.7		
High variates.......	149	164	126	164	162	178	140	178	14	202
Low variates........	77	86	84	77	39	45	36	36	41	112

average facet number was decidedly lower than that of the parents. In the male flies this difference was 26, and in the female flies 35. A count of the stock was made at the end of the experiment, and it was found that here the facet number had increased, the average for the males being 50 higher than that of the parents, and for the females 30. Previous to the time of the experiment, the stock had been kept in a room where the temperature was approximately 21°–23°, while, during the time of the experiment, it was kept at 19°–21°.

TABLE II.

EXPERIMENTS 1, 2, 3. FEMALES.

	Parents.				Offspring.				Difference Between Parents and Offspring.	Stock at End of Experiment.
Temperature........	(21°–23°)				27°					19–21
Catalog number.....	1	2	3	Total	1	2	3	Total		
Number of individuals...........	13	12	10	35	30	30	18	78		19
Facet averages......	75	89	82	82	47	54	42	47	35	109
Probable errors......				±2.2				±1.3		
High variates.......	100	127	98	127	88	102	86	102	25	146
Low variates........	43	52	65	43	31	30	26	26	17	54

Experiment 4 (Tables III., IV.).—Experiments 1, 2 and 3 indicated that temperature is an important factor in facet variation. To test this more fully one set of *Drosophila* was placed in an incubator, where the temperature was 28.5°, another was kept at room temperature, 22°, and a third set was placed on a cold plate at 17.5°. The offspring were then allowed to

TABLE III.

EXPERIMENT 4. MALES.

	Parents.				Offspring.			Differences in Facet Number Among Offspring at Different Temperatures.	
Temperature...........	22°				28.5°	17.5°	22°	17.5°–22.0°	22.0°–28.5°
Catalog number.........	4.1	4.2	4.3	Total	4.1	4.2	4.3		
Number of individuals...	10	13	12	35	80	29	17		
Facet averages.........	135	135	122	131	80	153	121	32	41
Probable errors.........				±3.4	±2.6				
High variates..........	192	174	180	192	161	202	186	16	25
Low variates...........	101	77	82	77	41	120	87	33	46

develop at these temperatures. In facet number, those which developed at room temperature gave an average of 121 for the males and 88 for the females, and differed but little from the parents; those which developed at 17.5° averaged 153 for the males and 149 for the females; and those which developed at 28.5° averaged 80 for the males and 45 for the females. In the above experiment, the electric current of the incubator was turned off for one and one half days so that the developing flies of the warm set were at room temperature for that length of time. In the counts, the effect of this may be noted as about one ninth of the flies is much higher than the rest, the developmental period being nine days at a temperature of 28.5°. The temperature of the cold plate was allowed to rise to that of the room twice for about three hours each time, so that the results are somewhat less striking in this experiment than in some others as regards the difference between high and low facet numbers.

Table IV.

Experiment 4. Females.

	Parents.				Offspring.			Differences in Facet Number Among Offspring at Different Temperatures.	
Temperature	22°				28.5°	17.5°	22°	17.5°–22.0°	22.0°–28.5°
Catalog number	4.1	4.2	4.3	Total	4.1	4.2	4.3		
Number of individuals	22	21	24	67	63	18	25		
Facet averages	84	75	76	79	45	149	88	61	43
Probable errors				±1.4			±1.0		
High variates	121	109	113	121	83	188	126	62	43
Low variates	43	57	44	43	20	106	62	44	42

Table V.

Experiment 4. 1. Males.

	Parents.[1]			Offspring.		Differences Between Parents and Offspring.	
Temperature	28.5°			28.5°	17.0°	28.5°–28.5°	17.0°–28.5°
Catalog number	4.11	4.12	Total	4.11	4.12		
Number of individuals	10	13	23	19	5		
Facet averages	89	72	79	66	135	23	63
High variates	140	120	140	86	148	54	28
Low variates	60	43	43	44	116	16	73

Experiments 4.1 and 4.2 (Tables V., VI., VII., VIII.).—Part of the offspring of experiment 4 which developed at different temperatures, was used as parents in similar experiments, only here flies developing at the room temperature were not used.

TABLE VI.

EXPERIMENT 4. 1. FEMALES.

	Parents.[1]			Offspring.		Differences Between Parents and Offspring.	
Temperature	28.5°			28.5°	17.0°	28.5°–28.5°	17°–28.5°
Catalog number	4.11	4.12	Total	4.11	4.12		
Number of individuals	13	17	30	21	4		
Facet averages	54	46	49	58	115	4	69
High variates	83	76	83	94	122	11	46
Low variates	47	31	31	40	106	7	75

TABLE VII.

EXPERIMENT 4. 2. MALES.

	Parents.			Offspring.		Differences Between Parents and Offspring.	
Temperature	17.5°			28.5°	17.0°	17.5°–28.5°	17.5°–17.0°
Catalog number	4.21	4.22	Total	4.21	4.22		
Number of individuals	13	11	24	31	9		
Facet averages	158	151	155	94	185	64	34
High variates	202	172	202	138	228	64	26
Low variates	128	131	128	50	136	78	5

TABLE VIII.

EXPERIMENT 4. 2. FEMALES.

	Parents.			Offspring.		Differences Between Parents and Offspring	
Temperature	17.5°			28.5°	17.0°	17.0°–28.5°	17.5°–17.0°
Catalog number	4.21	4.22	Total	4.21	4.22		
Number of individuals	13	11	24	31	9		
Facet averages	142	136	139	55	99	87	37
High variates	163	165	165	74	121	89	44
Low variates	108	106	106	28	65	80	41

The results obtained from all four sets are analogous to those obtained from experiment four. The results from the above experiments indicate that the temperature at which the parents develop has little, if any, effect upon the facet number of the offspring.

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[1] Development at room temperature for one and one half days.

Experiment 5 (Tables IX., X.).—This experiment is the most conclusive as a high temperature of 29° and a low temperature of 17.5° were maintained throughout. The male flies developing at the low temperature showed an average facet number three times as great as that of those developing at the higher temperature, the corresponding ratio for the female flies being 4.7. The average facet number of the male flies developing at 29° was 56, of those developing at 17.5°, 167; the average facet number of the females developing at 29° was 27, of those developing at 17.5°, 128. The parents of the above sets, developing at 22° showed little difference in the average facet number. In the males this difference was 2 facets, in the females it was 10. In both males and females the parents which had the higher facet average gave offspring with the lower average, these being

TABLE IX.

EXPERIMENT 5. MALES.

	Parents.			Offspring.		Differences in Facet Number at Different Temperatures.
Temperature..........	22°			29°	17.5°	
Catalog number.......	5.1	5.2	Total	5.1	5.2	
Number of individuals.	10	10	20	86	64	
Facet averages........	129	127	128	56	167	111
Probable errors........			±5.6	±0.9	±3.0	
High variates.........	200	211	211	98	226	128
Low variates..........	90	80	80	34	74	40
Coefficient of variation.			25	24	22	

TABLE X.

EXPERIMENT 5. FEMALES.

	Parents.			Offspring.		Differences in Facet Number at Different Temperatures.
Temperature..........	22°			29°	17.5°	17.5°–29°
Catalog number.......	5.1	5.2	Total	5.1	5.2	
Number of individuals.	12	12	24	71	75	
Facet averages........	97	87	92	27	128	101
Probable errors........			±3.0	±0.5	±1.5	
High variates.........	138	144	144	43	188	145
Low variates..........	34	53	34	16	83	67
Coefficient of variation.			25	21	20	

the ones that developed at 29°. The extreme variates (high, low) of the parents of the two sets are slightly greater in the case of the set which developed at the lower temperature, but as the greater difference here is only eleven facets, and as the difference between the variates of the two sets of offspring is as great as 145, it is certain that selection can play no great rôle in determining the facet difference of the offspring here noted.

Experiment 5.1 (Tables XI., XII.).—Parents of this experiment are the offspring of set 5.1 in experiment 5. In experiment 5.1, one set of offspring developed at 29° while another developed at 17°. The male flies developing at 29° gave an average which was 5 facets less than their parents which developed at the same temperature while the male offspring developing at 17° had a facet average which was 127 greater than that of the parents. The facet average of the female offspring was 11 greater for those developing at 29° and 120 greater for those developing at 17°, than that of the parents which developed at 29°.

TABLE XI.

EXPERIMENT 5. 1. MALES.

	Parents (from 5.1 Offspring).	Offspring.		Difference Between Parents and Offspring.	
Temperature	29°	29°	17°	29°–29°	17°–29°
Catalog number		5.11	5.12		
Number of individuals		7	21		
Facet averages	56	51	183	5	127
High variates	98	61	303	37	205
Low variates	34	38	127	4	93

TABLE XII.

EXPERIMENT 5. 1. FEMALES.

	Parents (from 5.1 Offspring).	Offspring.		Difference Between Parents and Offspring.	
Temperature	29°	29°	17°	29°–29°	17°–29°
Catalog number		5.11	5.12		
Number of individuals		16	26		
Facet averages	27	38	147	11	120
High variates	43	55	235	12	192
Low variates	16	27	111	11	95

A part of the offspring from set 5.11 was continued for three more generations at 29° (Table XIII.). The fifth generation gave an average of 63 for the males and 35 for the females. The males were lower and the females higher than the first generation (Experiment 5) which developed at 29°.

TABLE XIII.

THREE MORE GENERATIONS AT 29°—A CONTINUATION OF THE 5.II LINE.

	Males.			Females.		
Generation number	3	4	5	3	4	5
Facet averages	42	26	63	35	44	35
Number of individuals	17	7	55	9	25	52
High variates	58	86	130	46	56	49
Low variates	32	43	31	28	25	20

Experiments 14, 15, 16, 17, 18 (Tables XIV., XV., XVI., XVII., XVIII.).—In experiments 14, 15 and 17, the effect of temperature on the white bar-eye *Drosophila* was determined, and the results are fully concurrent with the results obtained from the work on the red-eye. Experiment 18 gives additional data for the red bar eye.

TABLE XIV.

PARENTS. EXPERIMENTS 14, 15, 17. DEVELOPED AT 27° C.

Males.

Catalog Number	14.1 15.3 17.2	14.2 15.4 17.3	14.3 15.5 17.4	14.4 15.6 17.5	14.5 15.1 17.6	14.6 15.2 17.1
Number of individuals	16	14	15	6	12	15
Facet averages	117	115	106	120	119	108
Probable errors ±	4.6	6.0	3.0	6.9	5.9	4.8
High variates	166	206	136	126	161	184
Low variates	56	60	72	82	82	75

Females.

Number of individuals	24	22	19	18	18	23
Facet averages	73	69	77	74	75	75
Probable errors ±	2.6	2.2	2.2	2.7	2.2	2.2
High variates	107	103	116	110	102	102
Low variates	44	44	57	47	38	40

These four experiments were made in order to determine the period of development when temperature is most effective. To determine this, five bottles with parents were placed at 29° and the developing flies in the different bottles were allowed to pass different fractions of their developmental period here. They were then removed to the cold plate at 15°, or to the room at

22°, and allowed to complete their development at these temperatures. One bottle was kept on the cold plate throughout the period of development, and another was kept at 29°. Removals from 29° to a lower temperature were made as follows: Number 2, after one half day; number 3, when the larvæ were about one third grown (2–2½ days); number 4, just before pupation had started; number 5, after most of the larvæ had transformed into pupæ. Number 6 was kept at 29°. In experiment 18, removals from room temperature (22°) to 29° were made, and

Table XV.

Experiment 14. Offspring. Part of development 29°—rest at 15°.

Males.

Catalog Number.	14.1.	14.2.	14.3.	14.4.	14.5.	14.6.
Number of days at 29°	0	½	2	4¼	6½	all
Developmental period in days	33	31	28	21½	10	8
Number of individuals	6	9	13	6	37	22
Facet averages	282	272	352	57	64	76
Probable errors ±	13	7.3	9	2	2.4	1.7
High variates	366	325	414	65	130	161
Low variates	240	217	280	52	45	39

Females.

Number of individuals	10	21	18	10	25	21
Facet averages	215	266[1]	231	56	56	59
Probable errors ±	11	16	5	2.4	1.4	1.1
High variates	288	650[1]	281	84	86	80
Low variates	147	190	171	43	39	48

Table XVI.

Experiment 15. Offspring. Part of Development at 29°. Rest at 15°.

Males.

Catalog Number	15.1	15.2	15.3	15.4	15.5	15.6
Number of days at 29°	0	½	2½	4½	9	all
Developmental period days				21	11½	9
Number of individuals	4	17	4	2	21	19
Facet averages	304	271	293	76	63	60
High variates	324	402	326	88	88	98
Low variates	290	161	248	65	40	42

Females.

Number of individuals	2	18	8	2	27	32
Facet averages	172	188	233	41	41	43
High variates	180	233	289	47	66	63
Low variates	164	130	178	36	33	27

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[1] Average includes female with 650 facets. She may be heterozygous for bar.

instead of using white bar-eyed flies, the red bar-eyed were used. As the parents were kept in the bottles for one day, the length of time the developing flies were allowed to remain at the initial temperature was reckoned from the middle of that day and not from the beginning of the experiment, in order to give the mean time of development at that temperature for all the eggs laid during that day.

Table XVII.

Experiment 17. Part of Development at 29°. Rest at 22°.

Males.

Catalog Number	17.1.	17.2.	17.3.	17.4.	17.5.	17.6.
Number of days at 29°	0	½	2½	4¼	7½	all
Developmental period days	12	12	12	11	8½	8½
Number of individuals	31	6	5	45	25	25
Facet averages	131	170	127	102	65	69
Probable errors ±	2.9	7.4	8.1	2.4	3.6	2.3
High variates	171	198	180	150	144	129
Low variates	99	125	96	36	32	42

Females.

Number of individuals	32	8	4	50	30	29
Facet averages	114	130	101	66	39	44
Probable errors ±	2.4	3.0	5.1	2.3	1.0	1.0
High variates	172	160	117	132	62	61
Low variates	80	112	80	32	25	31

Table XVIII.

Experiment 18. Part of Development at 22°. Rest at 29°. Red Bar-eye.

Males.

Catalog Number.	18.1.	18.2.	18.3.	18.4.	18.5.
Number of days at 22°	0	2½	6½	9½	all
Developmental period days	8¾	9¾	11	11¾	12½
Number of individuals	9	12	11	14	8
Facet averages	89	98	146	175	189
High variates	167	166	164	210	291
Low variates	49	64	65	128	124

Females.

Number of individuals	4	11	13	19	12
Facet averages	40	45	104	150	130
High variates	47	68	172	183	213
Low variates	35	35	52	120	101

In experiments 14 and 15 the effect of temperature is to be noted early in the larval period; in 17 and 18, which were changed from 29° to 22° and from 22° to 29° respectively, the effect of temperature is to be noted throughout the larval life. In no case was there any significant effect upon the facet number of the flies after the pupæ had been formed. The parent flies of the six different sets differed but little in facet number. In experiments 14, 15 and 17 each set of parents was used in each experiment. For instance, the parents of 14.1, 15.3 and 17.2 were identical. In experiment 18 the same parents were used throughout, being changed from one bottle to another.

Facet Change per Degree Change in Temperature.—With one exception, the number of facets per degree change in temperature varies from 5.2 to 8.9 and is fairly constant. The following is a list of facets per degree change, found by dividing the difference in facet averages by the difference in degrees centigrade:

Experiment.	Temperatures.	Facet Average Low Temperatures.	Facet Average High Temperatures.	Facet Differences.	Facet Difference. / Temp. Difference.
1, 2, 3, males	22° –27°	108	82	26	– 5.2
1, 2, 3, females	22° –27°	82	47	35	– 7.0
4 males	17.5°–22°	153	121	32	– 7.1
4 females	17.5°–22°	149	88	61	–13.5
4 males	22° –28.5°	121	80	41	– 6.3
4 females	22° –28.5°	88	45	43	– 6.6
5 males	17.5°–29°	167	56	111	– 8.9
5 females	17.5°–29°	128	27	101	– 8.0

The relation between the facet number and a ten degree difference in temperature is also very interesting. Is the number of facets increased two to three times per ten degrees decrease? The following table gives the results, n_t being the number of facets at the lower temperature and n_{t+10} at the higher temperature:

Experiment.	♀ $\frac{n_t}{n_{t+10}}$.	♂ $\frac{n_t}{n_{t+10}}$.
1, 2, 3	2.6	3.5
4	1.7	3.0
5	2.5	3.9
5.1	3.0	3.2
Average,	2.6	3.5

Size of Individual Facets (Plate I.).—The relation of the area of the eye to facet number was found to be constant for flies with an intermediate number of facets. This constant, for 43 individuals, was found by dividing the facet number by the area of the camera lucida drawing in 1/100 sq. in. In flies with a very small number of facets the relation does not hold; in very large eyes, the curved surface makes it difficult to obtain accurate areas. Leaving out of consideration these extremes, the change in facet number is accompanied by a corresponding change in the size of the eye, and there is no change in the size of facets.

DISCUSSION.

Observations upon the red and white bar-eye show that temperature is an important factor in determining facet number of the flies taken at random from a general population. Flies raised at a higher temperature, however, do not have a higher number of facets, but on the contrary, the higher the temperature of development, the lower the facet number. An explanation which may be offered presupposes the existence of a chemical factor or determiner which acts as an inhibitor of facet formation and that, at a higher temperature, the speed of reaction is much greater than at a lower. Then, according to Van't Hoff's law, with an increase of 10° C. the speed of the reaction should be from two to three times as great and the number of facets one half to one third times as large, and vice versa. An examination of the data shows a considerable degree of approximation to this condition. In the males with a 10° decrease the facet average is 2.6 times as great; in the females 3.5 times. Whether or not the high value, 3.5, in the females is due to the fact that bar-eye is sex linked and the female receives two chromosomes containing this factor, while the male receives but one, is a matter for speculation.

SUMMARY.

1. Temperature is an important factor in the determination of facet number in the bar-eye of *Drosophila*.

2. A lower developmental temperature results in a higher facet number, and conversely a higher temperature results in a lower facet number.

3. With each 10° drop in temperature between 29° and 15°, the facet number is increased on the average 2.6 times in the males and 3.5 times in the females.

4. The increase in facet number except at the extremes is

directly proportional to the increase in the area of the eye, and facet-size is therefore a constant within these limits.

5. Light, and amount of food, as they occur in these experiments are not important factors in the determination of facet number.

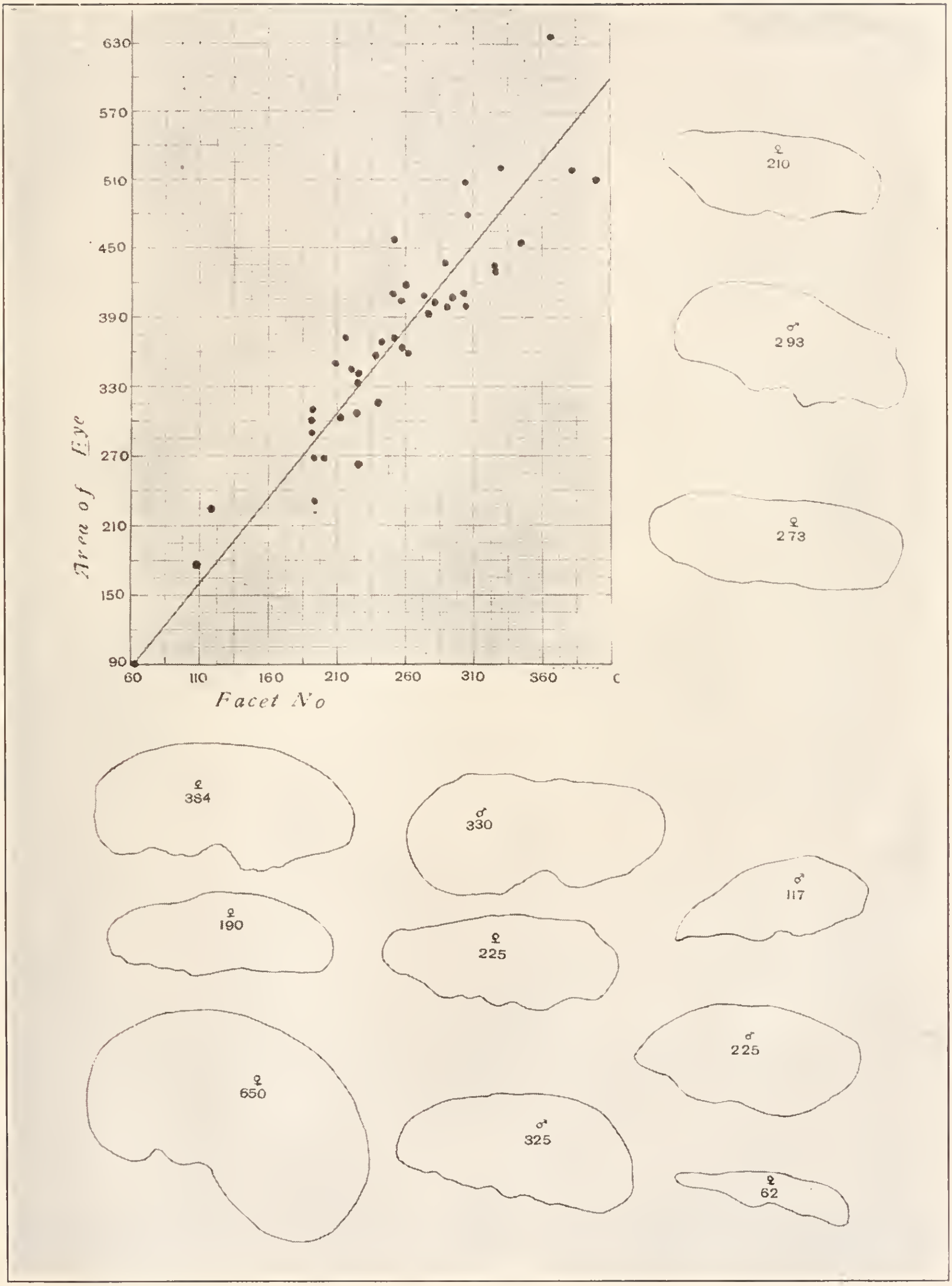
6. Temperature is effective only during the larval period.

Acknowledgment.

This work was done under the direction of Dr. Charles Zeleny and I wish to express my thanks to him for his interest in the problem and for his many suggestions while it was in progress.

Explanation of Plate I.

Relation of facet number to area of eye in white bar-eye. Distribution of forty-three cases around average represented by line. Camera lucida drawings of eyes, with facet number.


630
570
510
450
390
330
270
210
150
90
Area of Eye
60
110
160
210
260
310
360
Facet No
♀ 210
♂ 293
♀ 273
♀ 384
♂ 330
♀ 190
♀ 225
♂ 117
♀ 650
♂ 225
♂ 325
♀ 62

CIRCULATION OF THE CŒLOMIC FLUID IN A NEMATODE.

FREDERIC H. KRECKER.

The nematodes are not known to have a special circulatory system and it has been rather generally considered that they do not possess a circulating medium.

In some nematodes of the genus *Camallanus* taken from the rectum of a sunfish (*Apomotis cyanellus*) I noticed a flowing movement of the cœlomic fluid. It was blood red and could therefore be easily distinguished. Waves of the fluid passed from the anterior to the posterior end and then in the reverse direction.

The reversal of the circulation calls to mind the condition in ascidians. In them it will be remembered, there is a clearly defined circulatory system and the reversal of blood flow is due to the heart forcing the blood first in one direction for twenty or thirty beats and then, after a pause, sending it in the opposite direction. The only similarity in these two instances lies in the fact that in both of them the circulating medium is impelled first in one direction and then in the other.

Before describing fully what occurs in these nematodes it might be best to point out briefly such of their structural features as have a bearing on the phenomena. The specimens at hand are all females from 20 to 25 mm. long. As commonly happens among nematodes the uterus extends practically the entire length of the cœlom. It is so distended with embryos that in optical section it appears to be in contact with the body wall. As a matter of fact there is just enough space between the two for a thin layer of cœlomic fluid.

The circulation of the fluid is brought about by peristaltic contraction of the uterus. The ability of these constrictions to force the fluid along is due to there being more of it than can be accommodated in the narrow peri-uterine space. A sharp constriction of the uterus forms a comparatively deep depression which becomes filled with cœlomic fluid. This constriction moves as a wave lengthwise of the uterus and carries fluid along because the latter cannot easily escape.

The description of the entire process is as follows, beginning, for the sake of convenience, when the fluid leaves the posterior end. The peri-uterine space of this end is at this time filled with cœlomic fluid for approximately one-sixth of the worm's entire length. A peristaltic wave arises at the posterior end of the uterus and moves anteriorly. This is followed by another, then a third and sometimes a fourth. Each wave carries with it some of the fluid, the first taking the largest or primary wave of fluid, the third or the fourth, if there is a fourth, taking the least. The three waves all leave within seven seconds at slightly over two second intervals. After the last wave has left, only a thin film of fluid remains in the peri-uterine space. At about the time when the first of the peristaltic waves starts from the posterior end of the uterus, posterior to anterior waves of peristalsis are to be seen at its anterior end, each of them pushing along a little fluid. It was rather difficult to watch both ends of the worm at the same time and so my observations on what were practically synchronous occurrences may be subject to slight error. There are usually three of these peristaltic waves at the anterior end and then, with the fourth, comes the primary wave of fluid from the posterior. This wave arrives at approximately the time when the third wave is leaving the posterior end. The circulation is therefore a rather sluggish process since the time between the first and the third waves is seven seconds and the entire length of the worm is only twenty-five millimeters.

As soon as approximately one sixth of the cœlom at the anterior end has been filled with fluid, anterior to posterior peristalsis begins at this end and continues in the manner just described for the posterior end; a primary wave of fluid leaves, followed by a second and a third and sometimes a fourth, the first wave being the largest and each succeeding one smaller. There is this difference, however, between the processes at the two ends; when a peristaltic wave arises at the anterior end of the uterus it pushes cœlomic fluid both anteriorly and posteriorly instead of sending it all posteriorly. This movement in opposite directions occurs because the uterus does not completely fill the anterior end of the cœlom and consequently when it constricts it allows some fluid to move anteriorly. As a result, there is always a certain amount of fluid left at this end even after the last antero-posterior peristaltic wave has passed on. Another point of difference in the processes at the two ends is the fact that at the posterior end there is an instant's pause after the arrival of the fluid before it is sent back, while at the anterior end there is no pause, the peristalsis being immediately reversed. In view of this pause the posterior end can be considered as marking the beginning and the ending of the circulatory cycle. Seven cycles of the primary wave occur in two minutes. This rate was maintained by each of two worms in which the process was timed.

Frequently all of the secondary waves moving in a given direction have not reached the end toward which they are travelling before a primary wave starts toward them from that end. When this occurs the secondary wave keeps on moving until it meets the primary wave which then absorbs it and continues onward. When, as in the case of the posterior end, there is a momentary pause in the peristalsis of the uterus, the fluid does not necessarily come to rest throughout the entire cœlom, but secondary waves which may be moving keep on their course until they have reached the posterior end or until the primary wave from that end has met them.

Just why these secondary waves continue to move in this manner my observations did not make quite clear. The condition might be brought about by peristalsis from one end continuing until it met and was overcome by that originating at the opposite end. I am not prepared to say that this is the case, especially since, postero-anterior peristalsis begins at the anterior end almost synchronously with that at the posterior end. Possibly the impetus received from the peristalsis is sufficient to keep the secondary waves moving for a time even after peristaltic action has ceased.

As a wave moves along, a certain amount of the fluid composing it trails behind and comes to rest. This slipping back occurs because the uterus does not completely fill the cœlom.

The size of a given wave is maintained by carrying along the fluid in its path. As a result of the trailing fluid there is always some of it all along the cœlom. This residuum explains the arrival of small quantities of fluid at a given end with the peristalsis which precedes the arrival of the primary wave. The free play between uterus and body wall explains also why all of the fluid is not carried away from an end with the first peristaltic wave but is instead taken off in lessening quantities by several waves.

What relation the movement of the cœlomic fluid has to the well-being of the adult worm or its young depends upon the general function and composition of the fluid. Very little definite information has been published regarding this matter for the nematodes in general, and nothing, so far as I am aware, on the fluid of the genus *Camallanus.* It has been suggested that one of the functions served, in some cases at least, is to act as a medium for oxygen. If this be true the circulation in the present instance can be looked upon as of value in effecting a thorough distribution of the available oxygen.

There is some reason for believing that the circulation is of particular benefit to the young. Pointing to this conclusion is the fact that the peristaltic waves very effectively stir up the young and constantly bring different individuals into contact with the walls of the uterus and therefore nearer the cœlomic fluid.

From present knowledge it is rather hard to conceive what other purposes than those suggested would be served by such rhythmic constrictions of the uterus. Especially is it hard to see why there is a continual stirring up of the embryos bringing successively different individuals into contact with the walls of the uterus as is done in this case if it were not for some purpose connected with the surrounding medium. There is, however, a rather extensively held opinion that the cuticle of nematodes is impervious to all but the strongest fluids and even in some cases to strong formalin. If this were true the cuticle could possibly prevent absorption of oxygen. The view just mentioned is based on such examples as the vinegar eel, and on some instances in which adults of other species or their contained embryos have resisted preserving or fixing reagents for a considerable length of time. It is doubtful whether careful or extensive work would prove this imperviousness to be as general or as great as it is sometimes thought to be.

The fact that the cœlomic fluid is red points suggestively to an oxydizing function. In other nematodes having red cœlomic fluid the color is said to be due to the presence of hæmoglobin and this is therefore probably the condition in these worms. Since their host is an aquatic animal and since they live rather near the anus, it would be a comparatively easy matter for a certain amount of oxygenated water to come in contact with them. This being true, hæmoglobin in the cœlomic fluid could take up any available oxygen.

In dissolving any doubt regarding the function of circulation in these worms it would be materially helpful to learn how extensive the process may be among nematodes in general and under what conditions it occurs. There is so little color differentiation in the fluid of most nematodes that possibly circulation may have been overlooked in species where it does actually take place. In the present case the redness of the fluid in contrast with the light background of the tissues made movements of the fluid noticeable and easy to follow. If the process is primarily an oxygenating one it might be found more frequently among species internally parasitic or otherwise living where thorough distribution of a small supply of oxygen would be a matter of importance.

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THE INFLUENCE OF CERTAIN DUCTLESS GLAND SUBSTANCES ON THE GROWTH OF PLANT TISSUES.[1]

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That thyroid gland constituents and secretions contain substances which effect clear, and often far-reaching, influences on the metabolism and action of animal cells has long been recognized, both from observations of natural wild stock, and from experimental data. As a normally produced hormone, or as a substance introduced artificially, thyroid substances alter the norm of the metabolism of an adult tissue; in no less, but rather in more pronounced ways, thyroid tissue fed to growing embryos modifies their growth and differentiation.

Proof of the above has been furnished by numerous workers for vertebrates, especially for representatives of the mammalia and amphibia; and for insects and insect larvæ, as Northrop and Kunkel, respectively, have demonstrated. Protozoa also show a marked modification of their metabolic processes when they feed upon or absorb thyroid gland products, as indicated by the work of Nowikoff, Shumway, Budington and Harvey, and more recently by Chambers.

After noting that living substances in organisms of such widely different constitution and phylogenetic position seem distinctly susceptible to this hormonic material, the question arises,—Is thyroid substance a compound with special potencies over animal metabolism, or is all protoplasm amenable to its influence? Assuming a single origin of living material, has the wide divergence of the animal and plant kingdoms produced in the latter a type of protoplasm which may be immune to this glandular product, so foreign to plants in its place of synthesis?

This paper reports the morphological effects of thyroid constituents on the growth of the root-tips of the onion, *Allium*. To avoid seasonal eccentricities, the experiments were repeated three different years; and in this connection I am very glad to acknowledge much assistance from three senior students, Miss Helen F. Harvey, Miss Harriet M. Heeman and Miss Gladys Newman.

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[1] From the Department of Zoölogy, Oberlin College.

Methods and Results.

The experiments were carried out in mid-winter and early spring when the dormant period of onion bulbs is naturally terminating. Material was secured from the open market, from different dealers for successive trials; sound bulbs of medium size, suited to nicely cover the open tops of Naples staining jars of 45 mm. diameter, and 120 c.c. capacity, were selected; care was taken to discard any which already showed root growth.

The nutrient fluid used in the jars was Pfeffer's solution, made up according to the formula given in Duggar's "Plant Physiology."[1] Each experiment consisted of five or more groups of bulbs; to the nutrient fluid in jars supporting them was added desiccated thyroid gland (in tablet form put up by Parke, Davis and Co.) in the following amounts: Group I., 1 grain; Group II., 0.75 grain; Group III., 0.5 grain; Group IV., 0.25 grain; Group V. was the control, which grew on the nutrient solution alone. The solutions were stirred from time to time to secure homogeneity. Some experiments were carried out in a greenhouse affording approximately uniform temperature and humidity; but results obtained under these conditions did not differ from those conducted in the common laboratory environment.

A further detail may also be mentioned here; it was natural that solutions such as were used, containing organic substances, would gradually become infected with bacteria, no matter how sterile the ingredients at first. This feature of the procedure

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[1] Pfeffer's solution as used:

Calcium nitrate	4	grams.
Potassium nitrate	1	"
Magnesium sulfate	1	"
Potassium dihydrogen sulfate	1	"
Potassium chloride	0.5	"
Iron chloride	trace.	
Water dist.	5	liters.

would be of more importance in case the solutions were employed for prolonged periods; but in this work the bacterial factor never seemed to interfere with entirely healthy root growth. Indeed, Curtis has shown that, in some instances at least, a heavy infection of bacteria and fungus filaments about root sprouts on cuttings seems to be favorable to their better growth. His experiments covered many weeks, often months; deleterious effects due to carbon dioxide production in the solution may follow such infection after extended periods; but the factor is negligible in experiments limited to ten-day or two-week periods.

The following photograph is essentially self-explanatory and may be taken as typical of many. Roots grew somewhat in solutions of each different strength here mentioned; the very slight growth in the left hand bulb here figured would have continued to greater length after a time, but the proportionate lengths would have remained much as when the picture was taken. The apparent influence of the thyroid material is to retard growth rate.

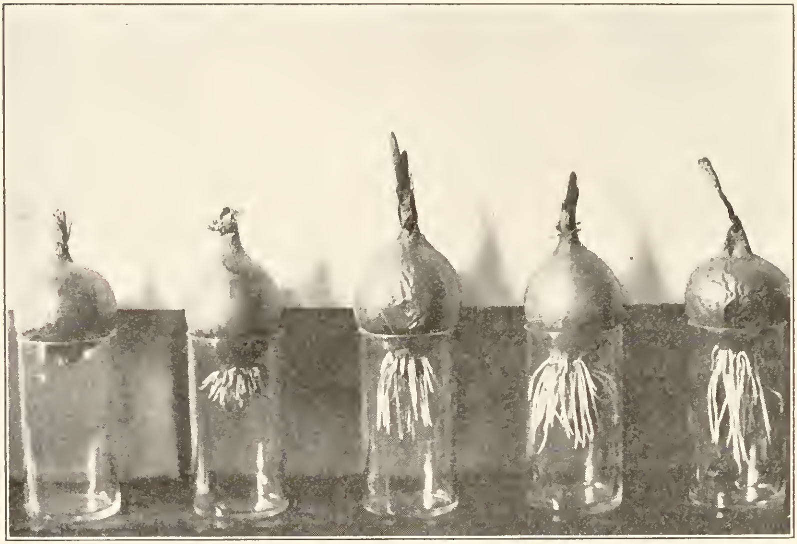

FIG. 1. Onion bulbs growing in Pfeffer's nutrient solution, in which is dissolved desiccated thyroid gland material, in parts of a grain indicated.

DISCUSSION.

The almost constant effect of abnormal amounts of thyroid tissues or extracts in the food or environment (or both) of developing animals has been to accelerate differentiation in premature ways. So too, one may interpret increased fission rate in Protozoa as indicating a similar effect, fission being a procedure characteristic of their attainment of adult physiological conditions. Such precocious metamorphosis of amphibian and insect larvæ is naturally coincident with small size.

The difficulties in estimating the effect of substances on plant tissues in terms of animal response and metabolism are obvious, and one should perhaps heistate to make any comparisons. However, all protoplasm obtains its raw materials through its permeable cell walls; and one may also go further and say that the root tips of bulbs, as they begin to grow, represent a kind of embryonic tissue with a minimum of specialization. In brief, the effect of thyroid substances on onion root-tips is to retard, or partially inhibit, size-growth; and this seems precisely the effect on embryonic animal tissue. In the latter, a hastening of physiologic and morphologic differentiation is also present; if the same be true in these root-tips, it naturally cannot be judged from external appearances, though one cannot deny that premature differentiation may be present. It is hoped to report on this point in a later communication.

Since the work of Marine and Lenhart, Morse, Swingle and others has made certain the earlier assumption that iodine is the most active principle in thyroid stuffs, a series of experiments in which only iodine was added to the nutrient solution was carried out. To make more significant and exact the comparison between such an experiment and those in which dessicated gland was used, KI was added in amounts based upon the accepted estimate that 0.01 c.c. of a saturated solution of KI furnishes the amount of iodine present in one grain of thyroid. Onion root-tips, sprouting in media with this iodine content showed no growth-rate which was specifically different from that of controls growing in nutrient solution alone.

Further sets of experiments were carried out with pituitary gland tablets, using strengths of 2, 1.5, 1 and 0.5 grains in 120 c.c.

of the nutrient fluid; supra-renal gland tablets were also used, in strengths of 1, 0.75, 0.5 and 0.25 grains in 120 c.c. of nutrient fluid; but there was no uniform effect of these substances, the appearance being that these substances so used have no influence at all on this particular plant tissue.

SUMMARY.

1. Growth of root-tips of *Allium* is retarded by the presence in their fluid nutrient environment of thyroid gland material. Retardation is approximately in direct proportion to the amount of thyroid substance present.

2. The presence of thyroid materials in the nutrient fluid in which *Allium* is sprouting does not modify the growth of the early leaves.

3. Iodine, used as KI, in amounts equivalent to that in thyroid substances provoking marked modifications of growth, has no appreciable effect on growing root-tips.

4. Pituitary substances up to two grains of the desiccated gland, and supra-renal substances up to one grain of the desiccated gland in 120 c.c. of nutritive solution have no effect on the growing root-tips of *Allium*.

5. While no general conclusion can be based on experiments limited to a single form, the indication is that thyroid constituents may influence the rôle of protoplasmic action in cells other than those of animal tissues.

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STUDIES ON THE ROTIFER ASPLANCHNIA EBBESBORNII, WITH SPECIAL REFERENCE TO THE MALE.

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Introduction.

The rotifers were collected in small rain pools, placed in aquaria with tap water and kept in the laboratory at room temperature, free from direct sunlight. The age of the cultures, when the present work was begun, varied from four months to two years. The rotifers appeared in the different cultures at regular intervals of about six to eight weeks.

The life cycle of *Asplanchnia* is as follows: females hatch from the resting or fertilized eggs. These females (first generation) produce parthenogenetically twenty to thirty offspring, which are likewise females. The latter reproduce parthenogenetically. All of the offspring of one parent, being either all females or males. Thus the females of the second generation may be called male producers or female producers. The life cycle of *Hydatina senta* as described by Shull, in most respects corresponds to the above description.

When the males become sexually mature, impregnation of the male-producing females occurs, and the impregnated females produce resting eggs instead of males or both resting eggs and males. The resting egg stage terminates the active period of each cycle and carries the rotifers to the beginning of the active period of the next following cycle. The active free-swimming period of each cycle continues from two to three weeks. The inactive or resting egg stage of each cycle varies from five to eight weeks. If impregnation of the male producers is prevented, no resting eggs are formed and the females continue to form males parthenogenetically.

If we consider the females that actually produce males the sum total of all male producing females formed, the percentage of the male producers would be rather low and remain constant regardless of external conditions. But if we include the resting egg producers (potentially male producers) in the sum total of the male producers the percentage under favorable conditions often reaches 90 to 95 per cent.

In this particular rotifer there are two kinds of resting eggs, which require fertilization before development begins. The one (single shelled) develops within the uterus of the parent with the same rapidity as the parthenogenetic forms and hatches out immediately when deposited. The other (double shelled) with the exception of a few early cleavage stages, develops after deposition.

No attempt was made to regulate the food conditions of the different cultures, except the adding of tap water at different times to counteract evaporation. The amount of food available for the rotifers varied greatly during the active periods of the different life cycles. Some of the cultures were almost entirely free from food of any kind, while other cultures were rich in euglenæ and unicellular plants or animals. It was found that the number of male- and female-producing females varied according to the amount and kind of food present. Scarcity of food favored the production of female-producing females. Culture rich in euglenæ and unicellular plants, favored a high production of male-producing females.

Experiments in mating and sex determination have been carried on extensively by Shull, Whitney and others but will not be considered here, since they do not come directly within the domain of the present investigation. However, some very suggestive features on sex determination present themselves in the study of the above rotifer. It is true that certain food conditions favor a high production of male-producing females, but immediately after impregnation, as stated above, these same male-producing females begin the production of resting eggs, or both male and resting eggs, which may alternate in the same female. Again it is not an unusual thing to find within the oviduct of the male-producing female embryo before birth, either mature male eggs or male eggs that have begun to develop, and if impregnation of these male producers occurs after birth, resting eggs instead are produced later. The question of sex determination from the standpoint of external conditions is rather an intricate problem to attempt to solve, since the same male-producing females are capable of producing simultaneously both males and female-producing females (from thin-shelled resting eggs) under the same food conditions.

Comparative Study of Male and Female.

The males with few exceptions as found in the different groups of rotifers are smaller than the females. The cleavage and early development in both sexes are quite similar. Previous investigators have based their results almost entirely on the study and development of the females and have not made a careful study of the degree of development and degeneration as it occurs in the male, more especially when compared with the conditions found in the female.

The development of the parthenogenetic female is represented in the series of diagram A, 1–15, and the parthenogenetic developing male in the series of diagrams B and C, 1–15. In case of the male the series B represents the condition, where degeneration of the male individual is very pronounced. Series C represents the condition of normal development, where only partial degeneration of the male occurs.

A. Formation and Maturation of Ova.

The ovary of female-producing male individuals and resting eggs and of the female-producing males are quite similar (Figs. 1 and 2). It is composed of a group of very small cells (ova), situated on the convex side of the vitellarium, near the point where the oviduct takes its origin. These small cells are uniform in size, and at regular intervals one of them begins its growth and becomes the mature ovum. Occasionally two begin their growth simultaneously. The growth is very rapid and the cytoplasmic granules pass directly from the vitellarium into the growing egg (Figs. 1 and 2). When growth is complete the egg is separated from the ovary and passed into the upper end of the oviduct where maturation occurs. The origin and formation of the male, female and resting eggs are quite similar. The rest-

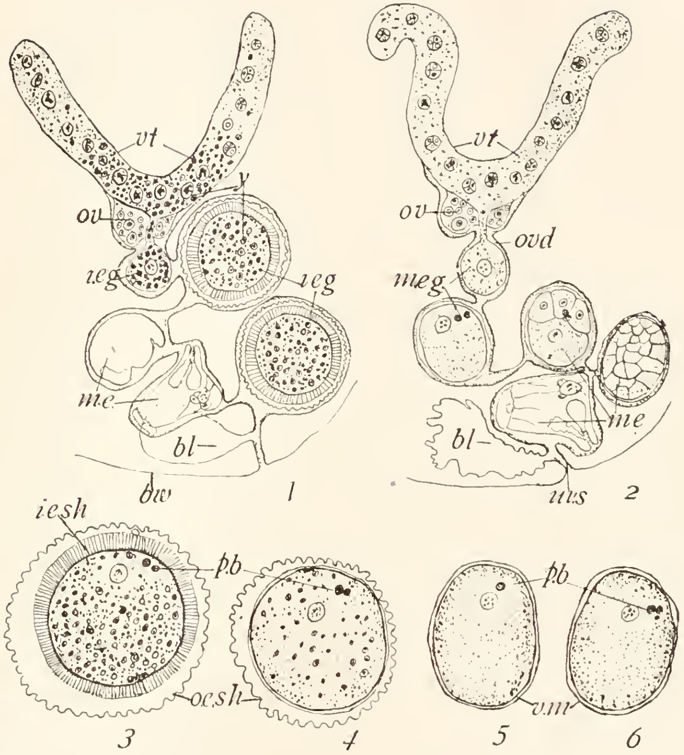

FIG. 1. Represents the reproductive system of a male-producing female, which shows the presence of both male embryos and resting eggs. Where male embryos and resting eggs alternate, the yolk is formed at intervals just preceding the growth of the resting eggs. The figure shows the passage of yolk into the forming resting egg.

FIG. 2. Represents the reproductive system of a male-producing female, which contains male embryos in different stages of development. The vitellarium is free from yolk and shows the passage of cytoplasmic granules into the forming male egg. One of the male embryos shows the condition at time of birth.

FIG. 3. A mature double-shelled resting egg, which is filled with considerable yolk.

FIG. 4. A mature single-shelled resting egg, which contains less yolk than the preceding. Its development is completed within the uterus and hatches immediately after deposition.

FIG. 5. A mature parthenogenetic female egg, which is entirely free from yolk. A single polar body is formed.

FIG. 6. A mature parthenogenetic male egg, which has the same structure as preceding. Two polar bodies are formed. All of the above eggs show the presence of a well-formed vitelline membrane.

ing egg (double shelled) is represented in Fig. 3. The outer shell membrane is external to the vitelline membrane and is formed by a secretion from the uterus. The inner protective membrane is formed from the cytoplasm within the vitelline membrane. Fig. 4 represents the single-shelled resting egg, which contains less yolk than the former. Two polar bodies are formed in either case. The female parthenogenetic egg (Fig. 5) is free from yolk and has a single polar body. The male egg (Fig. 6) with the exception of the two polar bodies is indistinguishable from the female parthenogenetic egg. The cytoplasmic content of the above eggs from the standpoint of quantity is about the same.

Impregnation of the male-producing female brings about a new condition in the formation and growth of the resting egg. The vitellarium instead of remaining transparent as in the formation of the parthenogenetic male and female eggs, becomes filled with numerous spherical yolk bodies, which pass directly from the vitellarium into the growing resting egg (Fig. 1, *r.eg*). These yolk-filled eggs are very dark, and unless fertilization occurs further development stops after maturation. The eggs deteriorate and do not give rise to males as advocated by some of the investigators on rotifers. The growth period of the female, male and resting eggs with the passage of cytoplasmic granules or yolk bodies from the vitellarium into the growing eggs, as well as the maturation stages can be demonstrated under the microscope in the living individual, the entire process requires about one hour. In this particular rotifer there can be no question as to the structural differences in the male and the resting eggs. The male eggs are always transparent, free from yolk and incapable of being fertilized. Whether the male and the resting eggs are the same or not before their growth period begins in the ovary of the male-producing females I am unable to say. There is a possibility of two distinct kinds of ova being present: the one which normally becomes the male egg, the other which requires impregnation before the growth period begins in the formation of the resting egg. The ova that become resting eggs never begin their growth unless impregnation occurs.

B. Cleavage and Gastrulation.

It is not my intention to give a detailed account of the different cleavages. A brief account of the more important stages of development, as they occur in the parthenogenetic female and male will be considered for the purpose of comparison in emphasizing the varying degrees of development and degeneration in these organisms.

The development of *Asplanchnia ebbesbornii* has not been worked out by previous investigators. Complete development (except of the double-shelled resting egg), occurs within the oviduct and uterus of the parent. The animals are extremely transparent so that it is possible to make a study of the various cleavage stages as they occur within the living body. The eggs are extremely plastic with a well-defined vitelline membrane (Figs. 5 and 6). The nuclear activities are distinct in the living cells, and the order, rate and direction of the cleavage spindles can be followed without any difficulty. Preserved material if stained, dehydrated, cleared and mounted in damar immediately after fixation, serves as a fine check in the study of cleavage. The cell lineage of *Asplanchnia Herricki*, from early cleavage to late gastrula, was worked out by Jennings. His entire work was based upon the study of preserved material. The rotifers were fixed in toto, the eggs dissected out and studied under the cover slip. With few exceptions he was unable to verify the presence of a distinct egg membrane. He seldomly found more than a single embryo in the same uterus.

The cell lineage of *Asplanchnia ebbesbornii*, for the most part is a confirmation of Jennings' results. The eggs are somewhat elliptical with the cleavage nucleus nearer the anterior end (A–C, 1). The first cleavage plane passes through the region of the polar body or bodies and divides the egg into two very unequal cells (A–C, 2). The second cleavage occurs at an angle of about forty-five degrees to that of the first, dividing the anterior smaller cell equally and the posterior larger cell unequally (A–C, 3 and 4). The four macromeres show considerable uniformity in the condition of their cytoplasmic structures. The region immediately surrounding the nuclei is more transparent and free from cytoplasmic granules. In the third cleavage the large cell *D* (A–C, 4) divides first. The ectomere d^1 is budded off in an upper anterior direction on the median dorsal side and covers the posterior ends of the macromeres *C*, *B* and *A*. The cells *C*, *B* and *A* before division are drawn out in an anterio-posterior direction and divide nearly equal. The ectomeres c^1, b^1 and a^1 are on the same level with their parent cells, as shown in the small diagram. During the fourth cleavage in the formation of the sixteen cell stage (A–C, 5) d^2 is the first cell formed, which is budded off from *D* in a dorso-anterior direction. The division of d^1 follows immediately. Next the macromeres *C*, *B* and *A* with their ectomeres divide, thus producing a twelve, fourteen and sixteen cell stage respectively. The embryo is now composed of four rows with four cells each (A–C, 5). Jennings describes the sixteen-celled embryo as being composed of four layers, each layer containing a single derivative of each quadrant. The first layer at the macromere end is designated as ventral and the cells are labelled accordingly. The number of layers increase with later cleavage.

The cleavage forms of the male and the female embryos at the sixteen cell stage are indistinguishable. The shape of the embryos, the size of the cells and the direction of the various cleavages (A–C, 5) are about the same. The only evidence we have to distinguish the male embryo at this stage is the presence of the two polar bodies, or more mature embryos in the same uterus. In the fifth cleavage all of the cells except the macromere *D* divide in a more transverse direction: thus doubling the number of rows on the surface. The inner ends of the cleavage cells withdraw towards the exterior and produce a central cavity, which is later occupied by the large cell *D*. Before the fifth cleavage is completed the anterior end of the cell *D* is partially covered by the cleavage cell immediately in front of it. The sixth cleavage is more irregular and doubles the number of cells in each of the eight rows.

Gastrulation is a double process, while the cells are passing posteriorly over the macromere *D*, the cell itself is moving into the central cavity. The entire process requires about fifty minutes and can be demonstrated in the living egg. The large cell *D*, which is now designated by the letter *E*, is destined to play a very important rôle in the future development of the individual. The derivatives of E give rise to most of the digestive tract, the reproductive system and a few muscle fibers. All other structures are derived from the surface cells, which form a rather deep epithelial layer around the central cell E.

Diagrams A–C, 6, represent late cleavage stages in which E has passed into the interior of the embryo and is no longer visible from the exterior. A little later stage (A–C, 7) than the preceding, shows the second cleavage of E as represented in an optical section from dorsal view. Diagrams A–C, 8, represent an embryo of either sex in optical section, as viewed from the left side with five large central cells derived from E. The small stippled space between the large cells and the outer epithelium represents the first indication of the presence of the future body cavity.

C. Differentiation of the Central Cells.

The large central cells $E^{2.1.1}$ and $E^{2.1.2}$ give rise to the reproductive organs, and the derivatives of the large central cells $E^{1.1}$, $E^{2.2.1}$ and $E^{2.2.2}$ produce most of the digestive system. The large central cells now divide very rapidly and fill the interior of the embryo (A–C, 9). These central cells as stated above become differentiated into two distinct regions, the entodermal (*st*) and the reproductive portion (*r.o.*), which shows the presence of darker granules and later gives rise to the reproductive organs proper. A few muscle fibers are derived from the central mass of cells, which are directly concerned in the movements of the reproductive organs and the stomach with the digestive glands

D. Embryonic Foot and Stomodæum.

The slight swelling at the lower posterior end (A–C, 9 ft.) marks the beginning of the foot, an embryonic structure. Later it becomes well developed in both sexes and shows the presence of two distinct toes, which no doubt persisted as a functional structure in its ancestors. The foot completely disappears before the birth of the individual. Diagrams A–C, 10 show the early stages in the formation of the stomodæum (*sto.*). The embryo is considerably curved upon itself and occasions the close proximity of the embryonic foot and the mouth. A distinct epithelium or hypodermis is present. The stomodæum in the male embryo is less prominent (B–C, 10), than in the female. The buccal pouch and pharynx is derived from this invagination. The posterior wall of the pharynx (A–C, 11) is more prominent in the female and contributes directly to the formation of the jaws or trophi. There is no indication of the formation of trophi in the male embryos.

E. The Rôle of the Entodermal and the Reproductive Cells.

The early differentiation of the derivatives of *E* bears a striking resemblance in the two sexes (A–C, 11). Two distinct regions are readily recognized. The upper and anterior part (*st.*) becomes continuous with the walls of the pharynx, and in the female (A, 12–13), gives rise to the œsophagus, the stomach and the digestive glands. The lower and more posterior portion (*r.o.*) becomes differentiated into the vitellarium, ovary, oviduct and uterus as indicated in the A series of figures. Beginning at the point of development as indicated in the series A–C, 11 the entodermal cells (*st.*), play quite a different rôle in the two sexes. In the male embryo (B–C, 11) the cells (*st.*) fuse with the wall of the invaginated pharynx as in the female, but do not give rise to any permanent structures. Later this entodermal group of cells lose their connection with the pharyngeal wall (C, 12), and are gradually utilized as food. The reproductive portion (*ts.*) gives rise to the testis and vas-deferens (C, 14). The male embryo as indicated in series B, 11–15, with the exception of the reproductive cells, undergoes an almost complete degeneration. In B, 11 the embryo corresponds to that of the normal male (*C*, 11), but later all of the non-reproductive cells deteriorate and are utilized by the embryo. When the sperm have reached their maturity, the embryo is represented by a large sperm sac (B, 15) within the delicate body wall or hypodermis. A portion of the trochal disc and cilia often persist.

In series C, 11–15, which represents the development of the normal male as it occurs in *Asplanchnia ebbesbornii*, degeneration occurs only in the digestive system. The early development however, is comparable to the conditions found in the female (A, 11). The cells from which the œsophagus, stomach and digestive glands are formed in the female, become independent of the pharyngeal wall in the male (C, 12–13), and persist more as a compact mass of cells, entirely free from any lumen (C, 14–15). The position of these cells within the body cavity is indicated in figures C, 11–15. This group of cells gradually becomes smaller and takes up a final position in the dorso-posterior region of the body cavity, where they are held in position by delicate muscle fibers. In extreme cases, when the male lives longer than normal, the cells completely disappear within the body cavity and no doubt serve as food. The reproductive organs are well developed (C, 11–15) and communicate with the urinogenital sinus by means of the vas deferens (C, 14). The urinogenital sinus is ciliated, which aids in the passage of the sperm. No attempt was made to represent more than a few of the muscle fibers within the body cavity. The excretory system which is well developed in the male is not shown.

The development of the digestive system reaches its greatest differentiation in the female as represented in series A. The stomach becomes a rather large strongly ciliated pouch, and communicates with the pharynx by means of a slender œsophagus, which is capable of considerable distention (A, 12–13). The stomach ends blindly. There is no indication of a rudimentary intestine in the developing embryo. The bladder (contractile vacuole) is formed by an anterior evagination of the wall of the urinogenital sinus.

Activities of the Male.

Varying degrees of degeneration are found in the males of *Asplanchnia ebbesbornii*, ranging from those without a functional digestive system, to those with all organs wanting except a large testis and the delicate hypodermis. The latter is little more than a sperm sac. In both instances however, the sperm are functional and capable of fertilizing the resting eggs. The methods of fertilization are rather unique. The normal free-swimming male is sexually mature at birth, and true intromission occurs at the urinogenital sinus (cloaca) with either kind of female. In the female-producing female, fertilization is ineffective and the female continues to reproduce parthenogenetically. In the male-producing female, after impregnation takes place, either all resting eggs are formed or male and resting eggs. The alternating of the two kinds of eggs cocurs more frequently when few sperm are present in the oviduct.

The uterus of the male-producing female may contain embryos ranging from early cleavage to late development (Fig. 2), but if at this point impregnation occurs, yolk spherules are formed within the vitellarium and all of the following eggs formed become true resting eggs. Maturation of the male and resting eggs occurs immediately after their separation from the ovary at the end of the growth period.

The sperm of the sexually mature males before their birth (Fig. 7, *m.e.2*) may be deposited into the uterus of the parent and bring about the production of resting eggs (Fig. 7, *r.eg.*) as above. In case of the extreme degenerate males (B, 11–15 and Fig. 7, *m.e.1*) the sperm sac and the delicate body wall break down, the sperm are set free in the uterus and the production and fertilization of the resting eggs follow. Again the sperm sac (B, 15) with the delicate body wall (degenerate male) may be deposited intact into the water and later the mature sperm are set free and finally get into the uterus of the different individuals. The independent entrance of the sperm is rather a simple process, since the urinogenital sinus and the lower end of the uterus is constantly being partially everted and inverted and during these activities the sperm make their entrance into the uterus. One of the peculiar activities of the normal male is the occasional eversion of the lower end of the vas deferens and discharge of the mature sperm into the water. The entrance of the sperm into the uterus independently of the male is a common method in this particular rotifer and makes possible the fertilization of a greater number of resting eggs. Those instances in which the male embryos and the resting eggs alternate in the same uterus, are often due to the later method, where sperm enter at different intervals.

The free-swimming males are rather scarce, less than one per cent. at any time, when compared with the total number of rotifers present in the different cultures. At the close of the active period of any one cycle, nearly all of the male-producing females, within a few hours show the presence of resting eggs within the uterus. These conditions are made possible by the independent entrance of the sperm.

In general the characteristics applying to the male rotifers

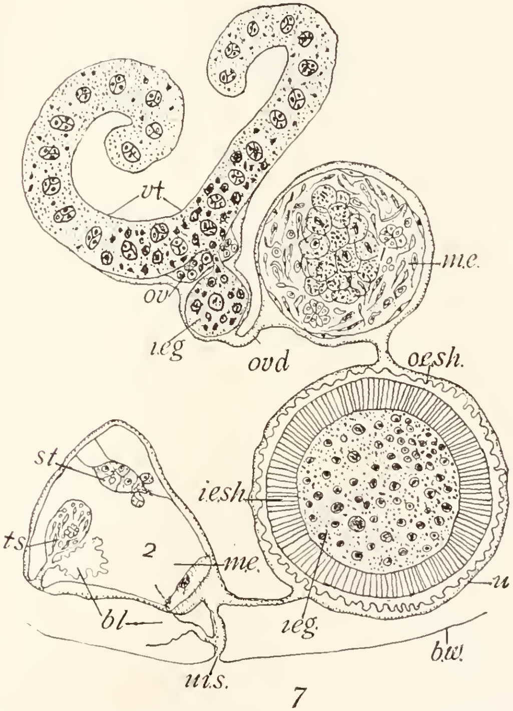

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Fig. 7. Reproductive organs of a male-producing female, which contains both male embryos and resting eggs; *r.eg.*, formation of resting egg; *m.e.1.*, male embryo which shows a case of extreme degeneration, with a very delicate body wall. The central mass represents a few somatic cells that still persist. No bounding membrane of sperm sac was formed, and the sperm as development progressed were left free in the body cavity. Later the somatic cells are all utilized and the body wall becomes the bounding membrane of the large sperm sac; *m.e.2* shows a completely developed male embryo at time of birth, with testis containing mature sperm; *r.eg.*, resting egg with two distinct protective shell membranes.

as a group are the following: diminutive size, the total absence of the mouth, of the pharynx, of the jaws, of the œsophagus, of the gastric glands and of the stomach and the intestine. The ovary is replaced by the testis. In some forms, however, like *Rhinops vitrea*, the male possesses all of the above mentioned points and does not show any indication of degeneration. In other forms as in *Polyarthra platyptera*, the male rotifer resembles a vorticella detached from its stem. Why degeneration is carried much farther in some species than in others is rather a puzzling problem. In *Asplanchnia ebbesbornii*, the varying degrees of degeneration are greater than in any previously reported species. Without the presence of the few actively free-swimming males, and more especially a knowledge of their embryonic development, the extremely degenerate males (B, 15 and Fig. 7, *m.e.*1) would in all probability remain unknown. It would not at all be surprising, if in some of the groups where males have not been reported, that extremely degenerate or parasitic males exist as described above.

COLUMBIA, MO.,
April 30, 1919.

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REFERENCE LETTERS.

a., anterior,
b.c., body cavity,
bl., bladder,
br., brain,
bu.c., buccal cavity,
b.w., body wall,
c., cilia,
c.o., copulating organ,
cp., corpuscle,
d., dorsal,
d.g., digestive glands,
ect., ectoderm,
ent., entoderm,
ft., foot,
i.e.sh., inner egg shell membrane,
l., left,
m., muscle,
m.e., male embryo,
m.eg., male egg,
mo., mouth,
oe., œsophagus,
o.e.sh., outer egg shell membrane,
ov., ovary,
ovd., oviduct,
p., posterior,
p.b., polar body or bodies,
ph., pharynx,
phw., pharyngeal wall,
pr., proctodæum,
r., right,
r.eg., resting egg,
r.o., reproductive organs,
st., stomach,
sto., stomodæum,
ts., testis,
u., uterus,
ur.s., urino-genital sinus,
v., ventral,
v.m., vitelline membrane,
vt., vitellarium,
y., yolk.

DESCRIPTION AND EXPLANATION OF SERIES OF DIAGRAMS A, B AND C.

All drawings were made with the aid of the camera lucida, under a magnification of about one hundred and fifty diameters and reduced one half. The drawings were made from either living or mounted embryos and checked. Stippling was adopted for sake of clearness. The text figures in the A series 1–15, represent the conditions found in the female. Fig. A, 15 shows the condition of the female embryo at the time of birth. The B series 1–15, represent the male embryos that have undergone extreme degeneration. Fig. B, 15 is characteristic of the end stage of development. The C series 1–15 gives the different stages in the development of the normal male with the degenerative digestive system. The buccal pouch and pharynx disappear before birth. Fig. C, 15 represents the condition of normal male individual at the time of birth.

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BIOLOGICAL BULLETIN

NOTES ON THE EFFECT OF X-RADIATION ON THE DEVELOPMENT OF CUMINGIA EGGS.[1]

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The energy of X-rays and of radium rays has been used by a number of investigators during recent years for modifying the normal course of development in living eggs. It has been sought to show by this means what efficacy these rays possess in altering vital properties of protoplasm, and to gain additional information concerning the normal as well as the abnormal reactions of living matter. One of us (Richards, 1914) had studied the effects of X-radiation upon early cleavage and development of *Planorbis*, a gasteropod, and it seemed probable that *Cumingia*, a lamellibranch, would offer interesting comparative data as well as give results of importance in themselves. For this reason the experiment herein described was performed.

Cumingia tellinoides is a form the eggs of which are very frequently used for experimental purposes at Woods Hole. The eggs and sperm are easily obtained separately, and may therefore be subjected to radiation either before or after fertilization.

The normal development of *Cumingia* is not as well known as is that of some other forms, but the more important features are described by a number of writers. Morgan, Jordan, Morris and others in their descriptions of experiments have presented normal conditions as well as results of experiments.

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[1] During the month of August, 1914, while working at the Marine Biological Laboratory at Woods Hole, the senior writer performed experiments to test the effect of x-radiation upon the eggs of *Cumingia*. This experiment yielded results which would have justified a much more extensive study of the behavior of these eggs under the stimulation of x-radiation, but circumstances have prevented its repetition. In spite of the fact that the experiment has not been followed up, and that the number of eggs worked upon was not large, it has been decided to make note of the results obtained, for they extend our knowledge of radiation effects.

During the course of the experiment eggs were preserved (usually in Bouin's fluid) for later cytological study. This material has been sectioned and studied in the Zoölogical Laboratory of Wabash College.

A. R.

In *Cumingia* eggs, Morgan ('10) found that normal development may follow in eggs that have undergone very considerable disturbances during experimental treatment, even "when the visible substances are unequally distributed, and are carried over into the blastomeres, redistribution being thereby prevented." Cleavage is persistently normal under very abnormal conditions, although very many factors may induce abnormal development, and the results manifest themselves only later. Abnormalities are especially apt to be caused by rough handling of the eggs in sea water after laying, injury to the egg membranes apparently being attended with serious consequences. "None of the visible substances are essential to the development of special parts of the embryo."

Polyspermy occurs in *Cumingia* eggs generally unless the sperm suspension be diluted before fertilization. This commonly is the cause of abnormal embryos, if it is permitted to take place to any extent at all. In our experiment the number of extra sperm, while not large, varied definitely depending upon the dosage of the X-rays, and therefore served a useful purpose in giving additional information as to the effectiveness of the rays.

The breeding season of *Cumingia* is at its height late in June and during the month of July. In August it declines and fewer eggs are laid by each female. The general impression prevails also among those who have worked with this form most that at the end of the season the egg is more easily injured, for instance by physical treatment, and so the percentage of eggs of a given lot which develops is less at that time. The number of eggs at any time which will develop depends upon their manipulation, for unnecessary handling with pipettes will cause low ratios.

At the time of fertilization the egg is in the metaphase of the first maturation division. Upon fertilization, maturation is completed in the usual manner.

The form and size of the first cleavage division is very constant and definite. The first division is unequal, the CD cell being much larger than the AB cell (Morris). The CD cell divides before the AB producing a 3-cell stage, then a 4-cell. After the third division cleavage becomes irregular, the D cell, or its derivatives, remaining for some time distinctly larger than the other blastomeres.

EXPERIMENTS.

In the set of experiments here recorded, all exposures to the X-ray tube were made at the same time; thus all three-minute irradiations were under the tube simultaneously All three-minute irradiations were removed at the same time, as were all fifteen-minute irradiations. Consequently there is in all the data presented no question whatever as to any varying amount of exposure for the same length of irradiations.

The irradiation intensity was therefore, a constant; hence, we may neglect it entirely and take the time in minutes as a measure of the amount of irradiation, not concerning ourselves quantitatively with the physical units involved. This is a much simpler method since when one attempts to calculate the amount of irradiation he enters a field entirely beyond the scope of this paper. What we wish to show is the comparative effect of different amounts of irradiation when conditions are identical.

Heat, the dilution of the suspension, and the other external factors were kept constant in order to neglect them and reduce any possible chance for error by a faulty technique or varying conditions.

Four series of experiments were set up. In the A series the eggs were irradiated immediately after fertilization; in the B series, sperm were radiated and then used to fertilize normal eggs; in the C series, eggs were radiated and then fertilized by normal sperm; in the D series, both egg and sperm were radiated before fertilization.

The first experiment was carried on to test the effect of short and long irradiations on the freshly fertilized egg. For this test fresh sperm and eggs were obtained from the individual animals and immediately mixed. Fertilization took place immediately. This lot was divided into three parts, one for control and two for experiment. All were allowed to develop under similar conditions. Samples of the three lots were fixed at intervals during the maturation and early cleavage divisions. A tabulated form of the observations is shown in Table I.

TABLE I.

Time After Irradiation.	Stage of Development.		
	Control.	A3 Radiated 3 Minutes.	A15 Radiated 15 Minutes.
20 minutes.	Completing 1st maturation division.	In second maturation division 1st polar body present.	Beginning 2d maturation division.
43 minutes.	40% in 2-cell stage rest dividing in 1st cleavage division.	Completing 1st cleavage division.	5% in 2-cell stage rest dividing.
60 minutes (1 hour).	36% in 2-cell stage. 14% in 4-cell stage.	25% in 2-cell. 25% in 4-cell.	30% in 2-cell. 10% dividing. 10% in 4-cell.
80 minutes (1⅓ hours).	25% in 2-cell. 17% in 4-cell. 8% in 8-cell.	20% in 2-cell. 20% in 4-cell. 10% in 8-cell.	25% in 2-cell. 20% in 4-cell. 5% in 8-cell.
325 minutes (5 hrs. 25 min.).	17% of those developing are free swimming larvæ.	10% of those developing are free swimming larvæ.	1% of those developing are free swimming larvæ.
385 minutes (6 hrs. 25 min.).	———	———	10% of those developing are free swimming larvæ.

Radiation at first serves to stimulate the mitotic activity of these eggs. The results are not as clear cut as in *Planorbis* where entire egg clusters can be observed without in any way disturbing the eggs in their normal environment. Nevertheless, it is clear here from Table I. that acceleration takes place at first in the irradiated eggs and that subsequent retardation follows as in the case of *Planorbis* where "the control, started at the time of the exposure goes more slowly than the experiment during the first two mitoses, but by the time the twenty-four-cell stage is reached the exposed eggs are progressing more slowly than it" (Richards, '14). Packard ('16) obtained similar results in *Arbacia*.

In a cluster of *Planorbis* eggs division takes place almost simultaneously in all the eggs. No such degree of uniformity is to be observed in *Cumingia*, but the data clearly indicate that the acceleration, while it persists longer than in *Planorbis*, just as surely gives way to a retardation, and that the longer radiation causes a greater retardation than the shorter. It would appear that greater exposure caused greater stimulation at first than the shorter, agreeing in this particular with the marine eggs which Packard ('16) studied, rather than with the fresh-water *Planorbis* and *Physa*.

From the 43-minute stage on the radiated eggs become slower in divisions, the percentage of free swimming larvæ in A3 being only a little over half the per cent. in the control, 325 minutes after fertilization. Only one or two per cent. of the A15 set have reached the free-swimming larval stage. However, from Table I. we see that one hour later or 385 minutes after fertilization it showed approximately the same percentage as the A3, 385 minutes after fertilization. This shows A15 at 325 minutes after fertilization is 20 per cent. behind A3 and approximately 35 per cent. behind the control in respect to time.

In figuring percentages of gain or loss during development and cleavage, we, following Packard, divide the difference in time required for the two sets to reach a certain stage by the time required by the control to reach that stage. Thus in the A series the control passed the 10 per cent. free-swimming larval stage several minutes ahead of A3. Now A15 entered that stage just 60 minutes later which is 20 per cent. retardation when compared with A3.

It was deemed inadvisable to carry the experiment farther for in both the control and the radiated lots a marked disintegration set in. It has been shown that disintegration may follow exposure to either radium (Packard) or X-rays (Richards, '15), but our radiation is hardly to be held responsible for the results of this experiment, since both control and experiment show it equally.

In Table I. reference is made to the percentage of the entire number of eggs which developed in the experiments. At the height of the breeding season a very large percentage of the number of *Cumingia* eggs fertilized are found to develop, usually over 95 per cent., it is stated. The precaution must be taken in that case to use dilute sperm in order to prevent polyspermy. The eggs used in this experiment, however, were obtained late in the summer and some polyspermy was permitted, for it was found, as will appear later, that the number of sperm entering the egg is itself influenced by irradiation. A count, made as carefully as possible, of the eggs of this experiment gives 54.25 per cent. as the average number developing. The data given in the tables refer to the number of eggs which developed, not to the total number of the experiment. These numbers were verified by counts of sections as well as of the entire eggs. Of the sections counted 53.5 per cent., and of the whole eggs, 55 per cent. were found to be developing, giving an average of 54.25 per cent.

The B, C and D series were governed by one control. Since all irradiations and fertilizations were at the same time this was made possible. Experiment B was performed to test the effect of two different lengths of exposure when sperm were irradiated and used to fertilize normal eggs. They were subjected to 3- and 15-minute exposure as in the A set. Samples of the control and of the experiment were fixed at varying intervals after fertilization. A study of these samples gave the following results:

TABLE II.

Time After Irradiation.	Stage of Development.		
	Control.	B3.	B15.
21 minutes.	Completed first maturation division.	Completing 1st maturation division.	Completing 1st maturation division.
31 minutes.	Early prophase of 1st cleavage division.	20% completing 2d maturation divisions and others developing are in the prophase of 1st cleavage division.	Completing 2d maturation division.
74 minutes.	Pro- and metaphase of 1st cleavage division.	Same as control.	Prophase of 1st division.
381 minutes.	20% of those developing are free-swimming larvæ.	25% to 30% of those developing are free-swimming larvæ.	10% of those developing are free swimming larvæ.

From this table we see that 20 minutes after fertilization the first sample shows no difference whatever between control and irradiated sets. The second sample taken 10 minutes later shows no apparent difference yet in the sets. The third sample, 74 minutes after fertilization, shows the short irradiation still the same as the control while the 15-minute irradiation is slightly behind. There is a marked difference in the percentages of free-swimming larvæ 381 minutes after fertilization. B3 shows an increase, B15 a decrease over the control.

Several observers have noted a stimulation to cell division when eggs have been fertilized by sperm subjected to an exposure of short duration. This observation can now be recorded for *Cumingia.* It is unfortunate that we have no samples of the experiment between the 74-minute and 381-minute stages of development in order to trace this through the cleavage development. The longer irradiation shows the expected retarded development, this length irradiation being harmful.

The C series consisted of radiated eggs fertilized by normal sperm. As stated before the control is the same as for series B. The following is a tabulated result from a study of the samples taken.

TABLE III.

Time After Irradiation.	Stage of Development.		
	Control.	C3.	C15.
21 minutes.	Completed first maturation division.	Giving off 1st polar body.	Slightly behind C3.
31 minutes.	Early prophase of 1st cleavage division.	In second maturation division, 1st polar body present.	In second maturation division.
74 minutes.	Pro- and metaphase of 1st cleavage division.	Early prophase of 1st division.	Same as C3.
381 minutes.	20% of those developing are free-swimming larvæ.	Nearly all dead; only one or two swimming larvæ.	Nearly all dead.

From this table it is evident that even a slight irradiation of the unfertilized egg causes retardation in the rate of cleavage and the development. That they do not develop shows that something has been interfered with in the metabolism of the embryo. Fertilization, however, appears normal in every respect and the polar bodies are given off normally.

The D series consisted of radiated eggs fertilized by radiated sperm. The control was the same as for B. Samples were taken similar to the other series. A study of the samples gave the following results.

Fertilization and maturation are slower in both irradiated sets than in the control. The shorter radiation, 74 minutes after fertilization shows a retardation over the control. The 15 minute

TABLE IV.

Time After Irradiation.	Stage of Development.		
	Control.	D3.	D15.
21 minutes.	Completed first maturation division.	35% giving off 1st polar body.	Same as D3.
31 minutes.	Early prophase of 1st cleavage division.	10% in prophase of 1st cleavage division rest completing 2d maturation.	All completing 2d maturation.
74 minutes.	Pro- and metaphase of 1st cleavage division.	Early prophase of 1st cleavage division.	2d polar body present; no prophase of 1st cleavage division.
381 minutes.	20% of those developing are free-swimming larvæ.	Nearly all dead; one third of living are free-swimming larvæ.	Nearly all dead; one sixth of living are free-swimming larvæ.

irradiation is behind the 3-minute exposure. The same handicap is present here as in the two previous series; there being no samples after this period until the free-swimming larval stage is reached. Results of development, 381 minutes after fertilization, however, show that the effect is quite similar to the series where the egg only has been irradiated.

It is worthy of note that here, as in the cases reported by the Hertwigs and by Packard, that the rays produce more effect upon the fertilized eggs than upon the unfertilized. (Compare Tables I. to IV., the A and the B, C, D series.) In general the effect of a short irradiation of the fertilized egg of *Cumingia* is a stimulation in the rate of cell division through the first and second cleavage divisions, after which time there is a retardation. A longer irradiation causes a less acceleration than the shorter treatment and markedly greater retardation and inhibition of growth. In the case of a short irradiation of the sperm (B series) no change whatever can be noted in the rate of division. In the C series where the unfertilized egg is subjected to a short irradiation, the effect after fertilization is a direct retardation of development and a complete inhibition of growth by the time the free-swimming larval stage is reached. There is apparently only a difference of degree in the extent of the injuries due to the two strengths of exposure.

The result of the D series is quite similar to that of the C series. It is evident that the irradiation of the sperm (B series) is not so harmful as a similar irradiation of the egg.

From a comparison of the number of eggs developing in the different series to that of the control a striking difference is noted. The following table will give by actual count the percentage of eggs developing in the different lots. We find no evidences in the nuclear structure of parthenogenetic development.

TABLE V.

Lot.	Percentage Developing.	Percentage Above Normal.	Percentage Below Normal.
Control (average)	54.25		
B3	60	5.75	
B15	50		4.25
C3	40		14.25
C15	40		14.25
D3	35		19.25
D15	35		19.25

For a sperm exposure of 3 minutes there is a net increase of almost 6 per cent. of the eggs developing. That is, there is a greater number of fertilizations due to a short exposure of the sperm. A longer exposure shows a decrease in the percentage of fertilizations which is below normal. The C series shows a net decrease of 14 per cent., there being no difference evident between the lots of eggs radiated for the longer or the shorter period. The D series is not, as might be expected, an average of the effect of B and C, but is a further decrease, the increase in the B series not being manifested in the D series. In fact the D15 eggs show a deviation from normal nearly equal to the sum of the deviations of the B15 eggs plus that of the C15 lot.

It appears, then, if this single set of observations be regarded as typical, that the percentage of *Cumingia* eggs fertilized is influenced by exposure of the eggs or sperm to X-radiation, and by the amount of exposure which they receive.

In connection with the effect of irradiation upon the percentage of fertilizations is the question of the effect upon polyspermy, for the same factor which would increase the percentage of fertilization would also tend to increase the number of supernumerary sperm to enter the egg. The results of actual counts of the number of extra sperm in the sections studied are presented in Table VI.

TABLE VI.

Lot.	Percentage Developing.	Extra Sperm.	No. Above Control.	No. Below Control.
Control (average)	54.25	1.59		
B3 (average)	60	3.40	1.89	
B15	50	1.20		.39
C3	40	2.58	.99	
C15	40	1.46		.13
D3[1]	35	1.00		.59
D15	35	1.50		.09

Fertilization, of course, is not simply a selection by the egg of one sperm nucleus out of several, for if that were the case it would follow that the increase in the number of sperm to enter the egg would insure a higher percentage of fertilizations. There is no definite relation between the number of eggs fertilized and the number with more than one sperm. B3 has the greatest number of extra sperm, and is also the highest in number developing; C3, however, is next in number of extra sperm, but the percentage of the eggs in development is below the control. These results show, since columns one and two of this table are inconsistent with each other, that at least one other factor (and, of course, many factors) are involved in fertilization which play no part in polyspermy. In the latter case the factors which cause the entrance of the sperm are the ones involved.

In interpretating the data collected in Table VI. care must be exercised. In the first place it is not possible to control the concentration of the sperm so that relatively many more may not have been added to C3 eggs, for example, than to D3. It is also possible that one set may have been handled more than another, although precautions were taken to eliminate these sources of confusion. Previous investigators have found a great deal of variation in the responses of different lots of *Cumingia* sperm and eggs during development, and the factors governing the behavior of any given lot are rather elusive and uncertain. It has already been pointed out in this paper that these experiments should be carefully repeated before a final acceptance of the interpretations here given.

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[1] Number of eggs counted was too small to permit an entirely trustworthy observation as to polyspermy.

The behavior of the extra sperm in the experiment, however, calls to mind the change in the activity of *Arbacia* fertilizin under the influence of X-radiation (Richards and Woodward, 1915). This secretion from *Arbacia* eggs possesses the power of activating sperm, and this property is affected by X-rays as follows: a slight radiation (2 min.) increases its activity, a long one (7 min.) lessens it, while an exposure of intermediate duration is practically without effect. Since a solution of *Arbacia* fertilizin in sea water (egg water) is definitely affected by irradiation, it is very probable that the substance which escapes from the eggs into the water would also be influenced by the irradiation before it passes out of the eggs.

The egg secretions of *Cumingia* and their properties have not been investigated, but Miss Cobb (see Woodward, '18, p. 464) "discovered that the eggs and egg water of *Cumingia* produce positive chemotactic response on the part of the sperm." Miss Sampson has found (unpublished) a secretion from *Cumingia* eggs which agglutinates its own sperm into very small groups of three of four sperm, and produces a very strong agglutination of *Arbacia* sperm.

In view of these facts and reasoning from the analogy, we are tempted to explain the facts shown in Table VI. by assuming that the fertilizin of *Cumingia* while yet in the egg was affected by the irradiation in such a manner as to bring about the results shown. The long radiations in each case where eggs were exposed, C15, and D15, show fewer extra sperm than the control which is to be expected if the long exposure decreases the activity of the substance as in the case of *Arbacia* fertilizin. Likewise in C3, a short exposure, more sperm entered into than the control eggs. This, too, is to be expected if the short irradiation activates the fertilizin. D3 represents the count of too few eggs to be dependable. In B3 and B15 where the sperm were radiated with the result that an increased number entered the egg after the short exposure and fewer than the control number are present, we may be dealing with a similar effect of the irradiation upon the sperm receptors (see Lillie, '19), although at the present time there is not even an analogy upon which to base such an assumption.

The irradiation of *Nereis* eggs (Packard) before fertilization frequently caused marked polyspermy, due to interference with the jelly formation about the egg. In *Nereis* the amount of jelly extruded from the egg at the time of fertilization is characteristically large; a similar explanation here is doubtfully possible in view of the smaller amount of jelly.

There is also other evidence that fertilization is affected by exposure to X-rays by means of a chemical change in the combining substance in the egg or the sperm. Richards ('15) and Packard ('16) have both shown the power of a short radiation in effecting a chemical change in proteids and some enzymes. Swartz ('08) found that exposure to radium is able to effect a chemical change in lethicin. Hertwig ('14) showed the direct effect of radiations of radium upon the chromatin content of the cell. These instances all serve to render plausible the explanation here suggested.

SUMMARY.

1. A short radiation of the fertilized egg of *Cumingia* stimulates cell division for a time then retards it. A longer radiation produces less stimulation and a greater retardation.

2. A short or long sperm radiation before fertilization if not too intense affects the rate of cleavage in no way.

3. A short radiation of the unfertilized egg causes, when fertilized with normal sperm, a retardation in development, the production of abnormalities and complete inhibition of growth before the free-swimming larval stages are reached.

4. A short irradiation of both sperm and egg before fertilization results in a direct retarding of cleavage and in the production of abnormal embryos, most of them never reaching the free-swimming larval stage.

5. Eggs fertilized by sperm that have been subjected to a short exposure to X-rays result in a greater percentage of developing eggs than would normally occur. A long radiation produces an indifferent result.

6. An exposure to X-rays of the unfertilized egg results in a lesser percentage of developing eggs upon fertilization by normal sperm. No difference is evident due to time of exposure.

7. Eggs and sperm each exposed to the X-ray before mixing

results in a lesser percentage of developing eggs than when the eggs alone were irradiated before fertilization. No difference is evident due to time of exposure.

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THE VAGINAL CLOSURE MEMBRANE, COPULATION, AND THE VAGINAL PLUG IN THE GUINEA-PIG, WITH FURTHER CONSIDERATIONS OF THE ŒSTROUS RHYTHM.

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Two years ago we recorded the results of a detailed study of the œstrous cycle in the guinea-pig. A rather full description of the histological and physiological changes which take place in the ovary, uterus and vagina during the "heat period" was presented. We emphasized particularly the importance of changes occurring in the microscopic composition of the vaginal fluid as indicative of the exact conditions in the uterine wall and ovarian follicles at corresponding moments.

Since that time we have somewhat extended the analysis of these phenomena. It has been found that a membrane covering the orifice of the vagina furnishes a most valuable and simple means of diagnosing certain periods in the œstrous cycle. This we have termed the "vaginal closure membrane." The exact moment of copulation and the conditions in the walls of the vagina and uterus at this time have been carefully followed, along with a consideration of the formation and significance of the vaginal plug. In the present paper a discussion of these several topics will be undertaken.

Certain points in the literature will also be discussed, a more complete review having been given in the previous article.

1. The Vaginal Closure Membrane.

In the former communication attention was called to the fact that "the external vaginal orifice, which during the period of œstrous activity is more or less open, actually showing in many cases a little fluid or some blood, closes and becomes less accessible after the period." During ovulation the vagina is open, but the fact of its being open is not unmistakable proof of the time of ovulation unless the open vagina also contains what was described as second or early third stage œstrous fluid.

At that time the method of closure of the vagina following the œstrus was not explained nor was its actual significance fully appreciated. The vagina is now found to be closed by a remarkable cellular membrane and in a very definite way.

The external orifice of the vagina is crescentic in shape and the urethral opening lies in front of it in the mid line. The anterior and posterior lips of the crescent-shaped opening come together, and a delicate epithelial membrane grows over the opening and unites the lips. This occurs shortly after the heat period in females that have not copulated and in those that have copulated the closure follows the expulsion of the vaginal plug, a process to be considered beyond. The closure begins at the tips of the crescent-shaped opening and progresses toward the midpoint. The lips do not approximate so intimately at the midpoint and the membrane here seems to be under more tension than at other parts, even after the entire orifice has closed. The opening of the orifice by a tearing of the epithelial membrane begins at the strained middle part and extends from there laterally until finally the vaginal lips are freely separated. The midpoint is, therefore, the last to close, and the first to open as a general rule, although at times the opening may begin at either side of the midline.

The epithelium completely unites the lips of the vagina so that nothing can escape from or enter into the vaginal lumen without tearing this closure membrane. Such a membranous closure of the vaginal orifice is unknown to us in any other mammal. In many species the sides of the vaginal opening may be approximated or cemented together by some hardened fluid or secretion so that the lips are not readily pressed apart, but a membranous growth closing the orifice after each heat period is apparently unique.

This membrane also completely closes the vaginal opening throughout pregnancy and only becomes ruptured when the vulva swells shortly before parturition.

Such an obstruction or closure of the vaginal lumen at once suggests the hymen of the human vagina. But this, of course, is quite a different structure in its origin as well as in its later history. The closure membrane in the guinea-pig not only exists in the young immature animal but is regularly destroyed before and reformed after every heat period that takes place during the life of the female. The formation or growth of this membrane might also be compared in some respects to the membranous growths tending to extend across and close the pharynx and other canals under pathological conditions.

The membrane is thin and delivate in structure and when stretched by slightly pressing apart the lips of the vagina with the fingers it is seen to be almost transparent, the outline of the vaginal lumen showing through. The closure membrane is of the same glossy appearance as is the surface epithelium covering the region of the vaginal lips with which it is continuous. It is composed simply of stratified squamous epithelium which has grown from the borders of the lips over the orifice and contains no vessels or blood.

When the membrane is torn or broken by accident during the diœstrum, or period of sexual rest, it reforms sometimes within a day, or within a few days, and remains until the beginning of the new period of heat or œstrus. A recognition of this membrane is then a great convenience in determining the onset of the œstrus in a group of female guinea-pigs. Daily smears of the vaginal fluid are not now necessary to find when the œstrus is about to begin in animals examined for the first time and whose rhythm is therefore unknown.

Although the presence of the closure membrane is a definite aid in recognizing the condition of the œstrous cycle, it must be remembered that this membrane often persists up to the first stage of œstrus, at which time the lumen of the vagina is filled with a mucous fluid and first stage cells. This is actually the "heat" time and the normal moment for copulation as we shall explain below. When the closure membrane still persists until the vaginal lumen is so filled it may be distended and rounded out resembling a blister membrane on the point of bursting. Puncturing this the vaginal fluid oozes out through the break. As a general rule, however, the vulva becomes inflamed and very slightly swollen immediately before œstrus and the stretched membrane breaks. Thus the membrane has reached the breaking point or has actually broken at just about the time the female is in heat and ready to copulate.

While the presence of this membrane is a reliable index of the œstrous condition, the open vagina, or its absence, is by no means indicative of the œstrous state. Although the vagina is always open during what we have termed the second and third stages of œstrous, and, therefore, at the time of ovulation whether copulation has taken place during the first stage or not, it is nevertheless frequently open at other times. It is not permissible to assume that the open vagina indicates a state of heat or the time of ovulation in a guinea-pig. Only when the open vagina contains fluid showing on examination the cells described as second or early third stage is the ovary almost exactly in the condition of ovulation. It may be stated parenthetically that after long experience one is able as a rule to diagnose the stages of the vaginal fluid by slight difference in color and consistency without microscopic examination.

Finally, then, when the vagina is open one may only be certain of the uterine and ovarian conditions by examining the contents of its lumen, but, on the other hand, if it be closed by this membrane one may be certain that the time of the new ovulation has not yet arrived.

2. The Time and Manner of Copulation and the Conditions in the Reproductive Organs of the Female at this Moment.

It is well known that female guinea-pigs in common with other animals of their class, and in fact most mammals, have a definite limited time during which they accept the male, the so-called "period of heat." This period, very slightly revealed by external signs at the mouth of the vagina, but chiefly by the act of copulation has been the starting point in all previous studies on the reproductive activities of the guinea-pig. In order to prevent the modifying conditions of pregnancy following copulation, various operations have been resorted to, as in the case of some of Loeb's experiments. Such operations might complicate or even vitiate the results which follow.

In the present account we wish to describe the exact moment at which copulation takes place during the sexual cycle and to show definitely the conditions of the vagina and uterus at this moment. From the condition of the vagina or uterus the ovarian condition is readily estimated, as we have shown in the former paper. After determining the exact œstrous condition of a female at the moment she is ready for copulation we may then recognize a corresponding moment in any female by an examination of the vagina without the necessity of introducing the male or permitting copulation to occur.

In order to designate the copulation time exactly, we must review briefly the characteristics of the four very clearly defined stages of the œstrus or "heat period" proper. During stage one the uterine epithelium swells, the cells becoming distended with an abundant mucous secretion which very soon pours into the lumen and reaches the vagina. At this time a desquamation of the epithelial cells from the lower part of the vagina also begins. The second stage shows a great accumulation of leucocytes below the uterine and vaginal epithelium with a slowly progressing desquamation of epithelial cells. The third is the stage of exodus of the leucocytes, myriads of them coming through the epithelial lining of the walls of the uterus and vagina, with an accompanying extensive destruction of the epithelium. During the fourth stage the broken down epithelium falls away in masses and at the same time a regeneration of epithelium takes place beginning from the mucosa of the uterine glands.

The Graafian follicles of the ovary rupture and ovulation occurs at the end of the second stage or the beginning of the third stage, while during the fourth stage the recently ruptured Graafian follicles are already well under way in their development into new corpora lutea.

A recent abstract by Long ('19) seems to indicate that four similar stages may be recognized during the œstrus in the rat, and that these stages agree almost exactly in significance with the comparable ones in the guinea-pig: Ovulation also occurring in the rat at the end of the second or beginning of the third stage.

During our initial investigation we made no attempt to locate the exact moment of copulation and, of course, did not describe the corresponding vaginal or uterine stage. The description by Loeb ('14), however, of the great leucocyte migration in the wall of the uterus twelve to twenty-four hours after copulation would indicate that the true "heat" or copulation occurs before the beginning of the destructive changes in the uterus, and this we now find to be true. Here and in the following section we wish to point out just how the uterine changes seem to be associated with the act of copulation, the retention of the sperm in order to insure the fertilization of the eggs, and after this the means of ridding the vagina of the excessive seminal accumulation.

A number of females have been placed with males while in one or another of the above mentioned four stages, as well as during different times of the diœstrum, or interval of sexual rest. The results show that a copulation is never accomplished except during the first stage of œstrus about twelve hours before the second stage begins. Long ('19), also finds copulation to take place in the rat during the first stage. At this stage in the guinea-pig the vagina contains a clear, foamy, saliva-like fluid in which desquamated epithelial cells of the first type are present. Differing from all other stages and times there are now no leucocytes to be found in the vaginal fluid,—compare our former Figs. 1 and 2 with Figs. 5 and 6. Even during the resting period the vagina contains some mucus but this is very scant and filled with many leucocytes, being pus-like in appearance and consistency.

To locate even more accurately the normal time of copulation the first stage may be subdivided into two shorter periods: a preparatory interval, the early beginning of the first stage, when the vagina is almost dry and contains only a scant amount of loose cells of the first type; and, second, what may be designated the true first stage, a more advanced period when the frothy mucous secretion has already begun to accumulate. Copulation takes place during this second phase of stage one and never during the first.

During stage one the vagina is characterized by two important conditions, both of which contribute to the success of copulation. In the first place the existence of a mucous secretion evidently facilitates the act of copulation. This abundant frothy mucous accumulation is limited to the first stage, particularly to the time when copulation takes place, and it never occurs in the vagina at other times. The second contributory condition is the complete absence of leucocytes in the mucous fluid. The leucocytes begin their migration through the epithelium of the uterus and vagina at the end of the second stage. They are extremely abundant in the lumen during the third stage, while from this time on they are found in the uterus and vagina in smaller or larger quantities up to the approach of the next first stage. Two days before the first stage begins leucocytes are still plentiful, but from this time first stage epithelial cells gradually become more abundant and the leucocytes decrease in number until finally when the first stage has actually arrived no leucocyte exists in the vaginal fluid. The mucous content of the vagina during the first stage hence lacks the pus-like appearance of the vaginal fluid of the "intermenstrual" time and is clear and foamy.

The absence of leucocytes from the vaginal lumen at the time of copulation is important, since, if present, they might by their dissolving powers or phagocytic action exert an injurious effect on the spermatozoa and thus interfere with their normal function. Later a special purpose of the leucocytes seems to be to destroy the excess of spermatozoa remaining in the uterus. This frequently occurs by an interesting process of phagocytosis. A leucocyte comes in contact with a spermatozoön which with its tail is longer than the leucocyte. The leucocyte by stretching and contracting finally takes into itself the entire spermatozoön, the tail being wound in circular fashion within the cell body. The leucocytes, however, apparently accomplish most destruction by their dissolving or disintegrating action.

It seems that the migration of the leucocytes through the walls of the uterus and vagina, though not increased in extent, is accelerated by the act of copulation and the entire œstrous process is shorter than in non-copulated females. About six hours after copulation the third stage is in full development, while under virgin conditions a comparable stage is reached only after at least twelve hours from the time when copulation might have occurred. It may be said that copulation tends to hasten ovulation, or that the act itself may facilitate the bursting of the Graafian follicles, which is a very old conception.

The act of copulation is short, lasting a few seconds only, while the preceding time of sexual excitement leading up to it is rather long. The male becomes excited by the presence of the female some time before she reaches the proper condition for copulation. A male after long isolation from females becomes sexually excited by the presence of any female irrespective or her sexual condition, and he invariably attempts to copulate. Nevertheless, the excitement of the male is not so strong nor prolonged when in the company of a female during sexual inactivity as with one during her sexual season. When the female is nearing œstrus the male is extremely excited and tries again and again to copulate, while at other times he soon tires and loses interest and ceases his aggressive behavior.

The male and female never fight during the long period of aggressiveness on the part of the male, which often lasts for many hours. The male tries to induce the female to copulate by irritation and excitement rather than by forcing her. The female may at times become nervous and attempt to bite the male, but an actual fight such as occasionally occurs between two males never takes place. No mating by force is observed; the consent of the female is necessary for the completion of copulation. Copulation is followed by a state of relaxation similar to that observed among mammals in general, and immediately afterwards both male and female may spend some time in cleaning their external genitalia.

3. The Vaginal Plug, Its Formation, Length of Existence and Manner of Discharge.

The spermatic fluid of the guinea-pig, especially that portion derived from the seminal vesicles, on entering the vagina of the female coagulates to form the *bouchon vaginal*, a rigid plug, filling the lumen of the vagina. This plug prevents the outflow of the sperm after every copulation. Such a vaginal plug has been described in many species of rodents and seems in general to be characteristic of this class of mammals. It was first observed in the guinea-pig by Leuckart in 1847. He correctly described it as a Pfropf (plug), formed by the coagulation of the secretion from the seminal vesicles and serving to fill the vagina and prevent the flowing out of the sperm after copulation.

Bischoff, in 1852, verified the observations of Leuckart and accepted his conclusions regarding the rôle of the vaginal plug in the copulation process. Reichert, 1861, differed with these two original descriptions in failing to find the formation of a vaginal plug after every copulation, and concluded that its presence was not a general phenomenon. Later, however, Hensen in 1876 brought new evidence confirming the observations of Leuckart and Bischoff.

Landwehr, in 1880, examined the seminal vesicles and found their secretion to contain twenty-seven per cent. of fibrinogen to which its coagulation reaction is due. Coagulation may occur as soon as the secretion of the seminal vesicles comes in contact with a small amount of blood.

Héron-Royer, 1881, observed the vaginal plug in *Pachyuromys duprasi*, but gave no satisfactory explanation of its formation. According to him the vaginal plug was formed in the vagina before copulation and was pulled out or loosened by the hooks on the penis during the act of copulation. These observations were entirely contrary to all earlier records, according to which the plug is formed after copulation and falls out some hours later. Blanchard made histological examinations of the vaginal plugs collected by Héron-Royer and found them to consist of two parts, a central, *partie centrale*, composed chiefly of great numbers of spermatozoa, and a peripheral part, *couche corticale*, formed of hardened mucus.

Lataste, in 1882, after examinations of the vaginal plug in the same species, *Pachyuromys duprasi*, came to quite different conclusions. He states that the vaginal plug, *bouchon vaginal*, as he termed it, is not formed as Héron-Royer claimed, before copulation, but immediately after, and in the same way as was known for other rodents. Regarding its function he accepted the old opinion of Leuckart that it serves to prevent the spermatozoa from flowing out of the vagina after copulation. He also mentions an instance in which a vaginal plug-like formation was found when there had been no previous copulation. From our observation on the œstrous discharge in the guinea-pig it is probable that this plug-like structure was nothing else than a concentrated accumulation of such a discharge, it having become unusually dense or dried out. In fact, as will be shown beyond, the superficial portion of the vaginal plug is actually the sluffed-off vaginal epithelium surrounding the coagulated seminal fluid. Thus the plug is partly of vaginal origin.

In later papers Lataste makes many contributions to the knowledge of the vaginal plug. In 1883 he described the vaginal plug in other rodents and pointed out that this formation was evidently not limited to a few species but was characteristic of the entire class.

Regarding the function of the vaginal plug, he slightly modifies his former position and concludes that its rôle is not only to prevent the sperm from flowing out of the vagina but rather by a filling up to push the sperm into the uterus. He extended the observation of Blanchard that the vaginal plug consists of two parts, differing in structure, a central core and a superficial envelope. He described the central part as consisting chiefly of the coagulated secretion of the seminal vesicles and also of a quantity of mucus, 1888*a*, while the superficial portion, *enveloppe vaginale*, was formed of stratified epithelial cells. The *enveloppe vaginale* is produced in the female by a rapid exfoliation of cells from the uterine glands and the vaginal walls on account of the irritating presence of the coagulated core. (His conception of the cause of the exfoliation is entirely incorrect.) The envelopment of the core by loosened epithelium from the vaginal wall serves to make easy the expulsion of the vaginal plug. This epithelial production he thinks is probably of a pathological nature and may be compared to the condition in women known as *vaginite exfoliante*.

These studies of more than thirty years ago by Lataste are in most respects surprisingly correct and it is only the nature of the process by which the outer epithelial envelope is formed with which we would materially differ.

Tafani, in 1888, described the vaginal plug in the mouse and found it to fall out about thirty hours after copulation.

Steinach ('94) found that the removal of the seminal vesicles from rats did not influence their sexual instincts or ability to copulate, but decidedly impaired the power of the male to fertilize the female.

Sobotta ('95) also has studied the formation of the vaginal plug in the mouse and found it present after every copulation. Histologically it consists of an homogeneous mass which is surrounded by an envelope of vaginal epithelium. Spermatozoa are more abundant in the central mass at its upper end or that portion near the uterus as the plug lies in the vagina. He confirms the observations of Tafani regarding the fate of the vaginal plug, finding that its surface gradually becomes soft and loose and the entire mass falls out of the vagina about twenty to thirty hours after copulation. Sobotta states that the vaginal plug in the guinea-pig falls out much sooner than in the mouse, being eliminated from the vagina within from four to nine and a half hours after copulation. The longer interval is approximately correct. He claims to have at times observed another copulation following the expulsion of the first vaginal plug.

Camus and Gley ('96) studied the coagulation process in the formation of the vaginal plug. They claim coagulation to be due to the influence of a prostatic enzyme, "vesiculase," upon the secretion of the seminal vesicles. The action of the prostatic enzyme is specific towards the seminal vesicle secretion of any rodent. The prostatic enzyme of a rat will coagulate the seminal fluid of a guinea-pig and vice versa.

Rubaschkin ('05) returns to the old opinion of Reichert, 1861, in claiming that the vaginal plug is not a constant formation in the guinea-pig following copulation. His statements are as follows: Bei der Maus (Sobotta), und nach Bischoff und Hensen auch bei Meerschweinchen bildet sich nach dem Coitus ein charakteristischer Vaginal-pfropf, der auf einen vorausgegangenen Coitus hinweist. Ich muss hier die Beobachtungen von Reichert bestätigen, dass beim Meerschweinschen ein solcher Vaginalpfropf sich meistens nich bildet. Von aussen konnte ich einen klaren Pfropf in der Vagina niemals erkennen; in einigen Fällen liessen sich einige Schleimstreifen bemerken, die aber ganz unregelmässig und nicht immer zu Tage traten. In seltenen Fällen wurden nach dem Secieren Vaginalpfröpfe gefunden, welche zum Teil aus verdichtetem Schleim, zum Teil aus Epithelzellen bestanden. Unter diesen Verhältnissen ist die Bedeutung des Vaginalpfropfs beim Meerschweinchen ganz nichtig, und am Anfange meiner Arbeit habe ich, durch diese Angabe Bischoff's irregeführt, einige Tiere verloren, weil sie zu spät getötet wurden.

Königstein ('07) described the vaginal plug in rats and agrees with the observations of Lataste, Tafani and Sobotta. He finds also the vaginal plug to consist of two parts, a central and a superficial. The vaginal plug contains in addition to the secretions of the male genital glands, mucus, detritus, many leucocytes, squamous epithelial cells in large numbers and a granular eosinphil staining secretion.

From this review the knowledge of the formation of the vaginal plug is found to be rather complete, although disagreements as to facts are expressed by several authors. It seems well established that the formation of a vaginal plug following copulation is a general phenomenon among the various species of rodents. The plug proper consists of a central core formed mainly by coagulated fluid from the seminal vesicles and this is surrounded or enclosed by a mass of flat epithelial cells, apparently derived from the vaginal wall. The coagulation of the seminal fluid may be due to the action of a prostatic enzyme although it is claimed that the coagulation occurs without the presence of such an enzyme. The vaginal plug as a whole falls out of the vagina a few hours after its formation.

On the other hand it is not clear from the literature just how or why the peripheral part of the vaginal plug, *enveloppe vaginale,* of Lataste is formed. And the manner and cause of the separation of the epithelial lining from the wall of the vagina are also unknown. These points could not be clearly understood without a knowledge of the changes occurring in the wall of the vagina and uterus during the œstrus, at which time copulation and the formation of the vaginal plug take place.

As we pointed out in our description of the œstrous changes, there is a stage in the cycle when immense numbers of leucocytes accumulate immediately below the epithelium lining the uterus and the vagina. From this position the leucocytes attack the epithelial cells and at the same time dissolve or destroy the connection between the mucosa and the subjacent connective tissue over extensive areas. This reaction is taking place a few hours after copulation during the latter part of stage one and throughout stage two of our description. A few hours later, during stage three, the leucocytes have made still further progress in their invasion of the mucosa and the destruction of its connection with underlying tissues. In certain sections of the uterus the entire mucosa filled with immense numbers of leucocytes is completely separated from the uterine wall and lies within the lumen, while in other regions the epithelium is loosely connected but still hanging to the wall. This disconnected and degenerating mucosa loaded with leucocytes breaks into small fragments during the fourth stage and is expelled from the uterus and vagina, while a new mucosa begins to regenerate from the mouths and the regions about the uterine glands and from the deeper layers of the vaginal epithelium. This is the fate of the mucosa when no copulation has taken place.

There is, then, no pathological "vaginite exfoliante" due to an irritation of the vaginal wall by the seminal fluid as Lataste thought. But a simple periodic œstrous breaking down of the uterine wall under leucocyte invasion, entirely independent of whether copulation takes place or not.

When copulation has occurred the loss of the epithelium follows a somewhat different course. Immediately after copulation the coagulated seminal fluid forms a mass within the lumen of the vagina and partly extending into the uterus. Around this mass the mucosa forms a close fitting envelope, thus preventing its early dislocation. The envelope serves to retain the plug in the vagina until the fourth stage of the œstrous cycle at which time the enveloping epithelium becomes completely separated from the vaginal wall by the dissolving effects of the leucocytes. The epithelium is now expelled as one continuous tube forming the cover around the vaginal plug instead of sluffing off in smaller pieces as occurs during the fourth stage when a copulation has not occurred. However, the vaginal epithelium may occasionally be shed en masse without copulation. In one striking case the epithelium was pulled out of the vagina as a conical sheath, enclosing the speculum that had been introduced for examination.

It is clear, therefore, that what was termed by Lataste the "enveloppe vaginale" is the layer of epithelium separated from the underlying connective tissue by the dissolving action of the leucocytes which invade the walls of the uterus and vagina at this time. It is also readily understood how the plug, after its short sojourn in the vagina and cervix of the uterus, is finally separated from its adhesion or tight connection with the wall and expelled as a mass from the vagina.

A possible function or effect of the vaginal plug in addition to those before mentioned has recently been suggested by Long ('19). He states that a stimulation of the cervix of the uterus in rats, by merely inserting a glass rod during stage one of the œstrus, prolongs the next cycle, and suggests that the vaginal plug may also act in this mechanical way. We have not tested the prolongation of the cycle in guinea-pigs following copulation without conception as compared with its length in virgin animals.

4. The Œstrous Rhythm.

In our earlier review of literature it was pointed out that the knowledge of the actual time of ovulation in the guinea-pig was decidedly inexact. Nothing scarcely was known of the periodic recurrence of the œstrus stages in a given female. In short the moment of ovulation in the guinea-pig was not available for accurate experimental purposes and no definite criterion or method had been devised for detecting the œstrous condition. And this was true in spite of a very long list of studies pertaining to the reproductive activities of these animals.

Reichert, as long ago as 1861, had found that the Graafian follicles rupture about nine to ten hours after copulation. This, in general, approaches correctness, but in cases where copulation has not taken place, or failed to be observed, such knowledge is of little consequence. Rubasckhin ('05) had more recently claimed that the vagina was open and the vulva somewhat inflamed ten to twelve days after parturition, but this is certainly too short an interval to indicate an actual return of heat. It must be remembered that the female guinea-pig goes into "heat" and accepts the male almost immediately after the delivery of her litter. This fact makes the length of Rubaschkin's interval still more improbable.

The most valuable and extensive investigations of the reproductive activities of the guinea-pig were those made by Leo Loeb ('11, '14). But here the data were derived almost entirely from examinations of the uterus and ovaries after their removal from the body of the female. While such studies did give a means of comparing the conditions found among different individuals at different times, and made it possible to estimate approximately the length of the sexual periods, yet this estimate could not be transferred with certainty to any one living individual. We further objected to Loeb's method of study since it failed to permit an investigation of the recurring œstrous periods in a number of unoperated females. The results of such an investigation would be most important in determining the influence of any unusual or experimental conditions introduced with intent to modify the intervals between ovulations or other periods of the sexual cycle. These are just such problems as Loeb had under consideration.

The entire literature showed that any such thing as a regular œstrous flow was completely undiscovered for the guinea-pig. It became necessary, however, for our studies to have an accurate knowledge of ovulation times, and to determine this, extensive investigations of the sexual cycle in the guinea-pig were undertaken. A simple method of examining the vagina of the living animal proved to be of the greatest value. Virgin females were selected and the fluid present in the vaginæ was taken daily by means of a small nasal speculum and cotton swab. This fluid was smeared on slides, stained and studied microscopically. The method is fully described in the former paper.

It very soon became evident that the vagina generally contained little or no fluid, but that periodically a great accumulation of mucus and cells was to be found. This excessive amount of mucus and cells is to be recognized as a typical œstrous flow. The constituent elements of the fluid change in their relative abundance in a definite manner from the beginning to the cessation of the flow. Four clearly marked stages, as mentioned above, could be separated by microscopic examination of the fluid smears.

These changes in the composition of the vaginal fluid were found to be associated with comparable changes in the structure of the epithelial walls of the uterus and vagina. And not only was this the case, but the changes in the vaginal fluid proved to be most reliable indices of definite processes taking place in the ovaries in connection with the rupture of the Graafian follicles and the expulsion of the ova. It is, therefore, evident that by an examination from time to time of this fluid, one may know the exact condition of the ripening follicles in the ovary and very nearly the exact moment of ovulation.

The œstrous cycles in a group of guinea-pigs were followed for a number of months in order to establish the normal periodicity or rhythm. The amount of variation that might exist in the length of the cycles in a given female was studied as well as the variations in cycle lengths among different individuals. An attempt was further made to discover any seasonal variations that might exist.

Only slight time variations were found in the periodic rhythm of a given female. For example, in one animal the record of six consecutive periods shows the œstrous flow to begin on the sixteenth day five times and on the fifteenth day once. In another case of seven consecutive periods the flow began on the sixteenth day six times and on the seventeenth day once. For further cases the reader is referred to the table given in our former paper.

There is only a limited variation in the length of the œstrous cycles among different individuals, ranging between fifteen and seventeen days in younger animals. In exceptional cases the period is slightly lengthened in older multiparæ, sometimes reaching eighteen days. These limits of fifteen and eighteen days for the lengths of the œstrous cycles have never been violated under normal conditions during the several hundred observations which we have now recorded. The method of examining the vagina for the closure membrane above described, and, in the case of its rupture, for the composition of the fluid contained within the lumen, renders these individual variations of no consequence in determining the exact "heat period" and time of ovulation.

Slight, if any, seasonal variations are shown by our animals. This may be due, however, to the uniformly warm temperature maintained in the breeding rooms during the winter months.

For a full description and photographs of the structural changes occurring in the œstrous fluid, the vagina, uterus and ovaries, the reader is referred to the original account.

After the publication of our results it was found that one of the last papers by Leo Loeb ('14), bearing on a related subject, had unfortunately been overlooked. We regret this, since a discussion of his methods and results would have been somewhat clearer in connection with our full consideration of the œstrus given in the previous paper than in the present connection. In earlier papers Loeb ('11) had completely failed to establish a definite length or periodicity for the sexual cycle in the guinea-pig. In the last paper, however, the length of the cycle was more nearly determined and a very thorough description was given of the microscopic changes taking place in the uterine wall during the heat period. Our independent account of the structural changes in the uterine wall fully confirms Loeb's description. But we are unable to agree exactly with the lengths of the sexual periods as estimated from his examinations of the removed uteri. In a still more recent article Loeb ('18) repeats his 1914 estimates and claims the lengths of the sexual cycles to vary between thirteen and a half and nineteen days.

In all cases Loeb's investigations had centered in a study of the sectioned uterus and ovary, thus necessitating their removal by operation or the death of the animal. Either procedure permits only one observation on a given female. No investigation of the uterus and vagina in the living animal had been made and no continuous observations on the consecutive cycles of given individuals were carried out.

As mentioned before, we recorded not only the structural changes of the uterus, but almost equally as marked changes in the wall of the vagina. And what we consider to be of still more importance from an analytical or experimental standpoint as a means of estimating the moment of ovulation, was the complete record of the changes in the microscopic composition of the vaginal fluid during the different stages of the sexual cycle. The removal and examination of this fluid is made without in any degree injuring the uterus or vagina and does not interfere with the further use of the female for ovulation and breeding records. This knowledge of the definitely changing structure of the vaginal fluid made it possible to study the œstrous cycles in many living females and reduced the time element of ovulation in the guinea-pig to a certainty.

We considered in a somewhat different manner the connection between the uterine reaction and the secretion of the corpora lutea, though essentially we share Loeb's ideas of the functions of these bodies. It was concluded that the duties of the corpora lutea are probably about what Beard ('97) long ago argued in his monograph on "The Span of Gestation and the Cause of Birth."

The development and the degeneration of the vaginal and uterine mucosæ were found to follow very closely the development and degeneration of the corpora lutea in the ovaries. The case was stated as follows: "The breaking of the Graafian follicles occurs during the œstrus as a result of congestion which began in the theca folliculi at about the same time as the congestion of the stroma of the uterus and vagina. And finally when the regenerative growth of the uterine mucosa sets in, the ovaries then possess new corpora lutea in an active state of differentiation which have been derived from these recently ruptured follicles." The presence of the new active corpora lutea suppresses the final steps in the development of the almost mature Graafian follicles in both ovaries, whether the corpora lutea be located in only one of the ovaries or both. When the corpora lutea become less active and their degeneration has proceeded to a certain extent, another ovulation may then take place. Therefore, the functions of the corpora lutea are probably, first, by their presence and activity to inhibit ovulation or to determine its time, and, secondly, to preserve the structure of the uterine wall and prevent its degeneration.

Loeb has attacked the problems of corpora lutea function in the guinea-pig in a more direct experimental way than have other investigators. Yet while studying the effects of corpora lutea removal on the length of the ovulation period, he has been handicapped by the fact that his animals, after the initial operation, were later killed for examination and thus were no longer available for a continuation of the experiment. Only one observation was obtained from any particular female. The effects on the lengths of the ovulation periods of the removal of corpora lutea or the application of its extracts could be investigated to great advantage on guinea-pigs in which the œstrous cycles are definitely known and followed through a number of consecutive periods. This could readily be done by the method before described. This method is also of value in locating the early stage of developing eggs and in making exact matings for studies on fertilization, etc.

Attention may be called to further slight objections that might be raised in considering Loeb's last paper. He studies the conditions in the structure of the uterine wall removed from females that had copulated shortly before, as well as, uteri from uncopulated females, and states, page three: "The sperm fluid present in the lumen of the uterus exerts a pressure on the surface epithelium and may thus contribute to the harmful influence of the leucocytes." This idea is incorrect since it may be clearly shown that the action of the leucocytes is equally as harmful in the destruction of the uterine epithelium during the œstrous period of virgin females.

In a similar connection Loeb also finds, page 11, that the number of leucocytes in the uterine mucosa is much smaller in animals that have not copulated. Again, page 16, "A few leucocytes can also be seen in the uterus of animals in which copulation had been prevented. . . . In such cases (non-copulated animals) also some degenerative changes occur in the uterine epithelium, but they are less marked than in animals which had copulated." These statements are not entirely in accord with our findings since there is no such marked discrepancy between virgin females and those that have copulated. Such conclusions are probably due to the fact that the uteri examined were not removed from the non-copulated females at the maximum moment of leucocyte migration and degeneration of the uterine wall (our "third stage"). Loeb had no exact means of knowing the comparable stages in copulated and non-copulated females.

The uterine mucosa of our virgin females may show leucocytes to be equally as abundant at comparable stages as the uteri from specimens after copulation. It must be recognized in this connection that each stage in the condition of the uterine wall during the œstrous is of short duration and unless the uterus be removed at a given time, the abundance and position of the leucocytes and the condition of the uterine wall will be changed. Chance was against Loeb's removing the uteri from the non-copulated females at the moment of maximum leucocyte migration, since he had no exact means of knowing when this would occur without having first observed copulation.

Active migration and accumulation of leucocytes may be observed in the entire absence of sperm fluid. The rôle of this fluid and the modification of the shedding or sluffing off of the vaginal and uterine epithelium in its presence was fully brought out in the discussion above of the vaginal plug.

Loeb's estimation of the ovulation times and uterine changes from microscopic examination of fixed specimens does not make it possible to know within a few hours, or even days, of the exact moment of ovulation in a given living individual. He states, however, on page 31, that to determine the effects of the removal of the corpora lutea on the duration of the sexual cycle, it "was necessary to determine the length of the cycle in the normal guinea-pig." Not only is this necessary, particularly in view of the wide variations Loeb finds in the normal sexual cycle among different individuals, but it is better or even necessary, to know the actual length and variations of the sexual cycle in the given specimen experimented upon. As evidence of the correctness of the last statement, we may cite Loeb's method and results in determining the normal cycle lengths. This was done "by observing the time of heat of a guinea-pig and by examining the uterus and ovaries at known intervals" (after removal from different individuals). Such examinations were made on many specimens that had to be either killed or operated upon. The following ovulation intervals were thought to be the normal sexual cycles, page 31, "We found the length of the sexual period to be usually sixteen to eighteen or nineteen days; sometimes the new ovulation may take place as early as fifteen days after copulation. In two exceptional cases we observed the new ovulation as early as thirteen and a half to fourteen and a half days." The sexual cycle, therefore, varies in length from thirteen and a half to nineteen days, a range of almost six days.

On this basis it is seen to be practically impossible to state within a day or so of when the next ovulation will take place in a female that has just passed the "heat period." And the "heat period" was very indefinitely known unless copulation had been observed. Thus, as a usual practice, in order to prevent pregnancy following a copulation used to prove the existence of heat, the oviducts were previously tied. Such a procedure might easily modify to some degree the sexual cycle and is inconvenient for further study of the animal.

Any significant experimental modification of the ovulation intervals could be readily detected by a simple examination of the microscopic structure of the vaginal fluids collected from a guinea-pig.

Finally, the "signs of heat" recorded from a report by Miss Lathrop, an animal breeder, are, according to our experience in examining and mating guinea-pigs, generally inaccurate and of little value for use in experimental studies.

5. Summary.

1. The œstrous cycle in the guinea-pig is very definitely limited in length. Ovulations follow one another every fifteen to seventeen days in younger individuals, while in old females the period is slightly lengthened, in exceptional cases to eighteen days.

2. These individual variations are readily controlled by the method of vaginal examination described in this and a former article, so that the actual moment of ovulation in any given female may be determined to within almost an hour of the rupture of the Graafian follicles.

3. During the period of sexual inactivity, the dioestrum, as well as during pregnancy, the orifice of the vagina is completely closed by an overgrowth of epithelium which we have termed the "vaginal closure membrane." This membrane ruptures just before or during the first stage of the œstrus in non-pregnant females and before parturition in the pregnant. It always reforms to close the vagina shortly after the "heat period" has

passed. The presence of this closure membrane is therefore positive evidence that the time of a new ovulation has not been reached. When the membrane is ruptured and the vagina open, the ovarian condition may then be determined by examination of the fluid content of the vagina as described. A knowledge of this closure membrane greatly facilitates the examination of females in locating the beginning of the œstrus.

4. Copulation takes place in the guinea-pig during stage one of the œstrous period and ovulation occurs, as we previously showed, at the end of the second stage or the beginning of the third. Long ('19) finds exactly the same to be true for the rat.

5. At the time copulation occurs, the vagina is filled with a clear foamy mucus, and this is the only time at which no leucocytes are present in the fluid. Both the nature of the vaginal fluid at this time and the absence of leucocytes contribute to the success of copulation and fertilization.

6. A vaginal plug is formed a few minutes after copulation, during the first stage, and remains in the vagina for only a short time, being expelled during the fourth stage of the œstrus.

The core or center of the vaginal plug is composed chiefly of coagulated seminal fluid. This is enclosed within an envelope of epithelial cells, being simply the sluffed-off mucosa from the wall of the uterus and vagina. This epithelial cover which is thrown off at every œstrous period is loosened or dissolved away from the uterine and vaginal wall by an enormous invasion of leucocytes which progresses from the first to the third stage. The breaking away from the vaginal wall of this enveloping epithelium causes the plug to fall out of the vagina during the fourth stage. After the expulsion of the vaginal plug, the mouth of the vagina is closed by the growth of the vaginal closure membrane.

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THE INDIVIDUALITY OF THE GERM-NUCLEI DURING THE CLEAVAGE OF THE EGG OF CRYPTOBRANCHUS ALLEGHENIENSIS.

BERTRAM G. SMITH.

Early observers of the process of fertilization described the meeting of the sperm-nucleus and the egg-nucleus, and their complete fusion to form a single zygotic nucleus, the primary nucleus of the embryo. Later it was found that in many cases, though apparently not in all, the two germ-nuclei merely become apposed without fusion. In tracing their further history it was shown, in certain cases, that the germ-nuclei give rise to two independent groups of chromosomes which separately enter the equatorial plate and whose descendants pass separately into the daughter-nuclei. "Later observations have given the strongest reasons for believing that, as far as the chromatin is concerned, a true fusion of the nuclei never takes place during fertilization, and that paternal and maternal chromatin *may* remain separate and distinct in the later stages of development—possibly throughout life" (Wilson, 1900, p. 204). At the present time there is much to warrant the belief that a fusion of maternal and paternal chromatin never takes place in the somatic cells, while in the lineage of germ cells nothing approaching fusion occurs until a pairing of maternal and paternal chromosomes, called synapsis, takes place in preparation for the maturation divisions; then for the first time maternal and paternal chromosomes are brought together in intimate and orderly union, in some cases amounting to fusion.

This conclusion, which is of the most fundamental importance since it vitally concerns the mechanism of heredity, is based largely on indirect evidence; for in almost every case apparently insuperable obstacles have been encountered in the attempt to trace the respective maternal and paternal chromatin-complexes through the resting stage of the nuclear cycle. In only a very few cases has the individuality of the germ-nuclei during this stage been demonstrated with any considerable success; the observations of Häcker (1892), Rückert (1895) and Conklin (1901) are of most importance in this connection and will be discussed later.

In the developing egg of the amphibian *Cryptobranchus allegheniensis* I have found material very favorable for the study of this problem. The early cleavage nuclei are invariably double throughout the entire resting stage, each consisting of two separate though closely apposed nuclear vesicles, and the origin of each vesicle from a single germ-nucleus has been clearly traced. Indeed, my observations indicate that typically the germ-nuclei maintain their individuality throughout cleavage, though certain irregularities, real or apparent, occur with increasing frequency in the later segmentation stages.

TECHNIQUE.

Most of the sections used in making the observations recorded in the present paper were cut from eggs killed with bichromate-acetic-formalin. This mixture, made up in the right proportions (Smith, 1912, I.), preserves the form of the egg perfectly in all stages, enables it to be sectioned by the paraffin method, and gives very satisfactory histological and cytological results. For comparison, sections were made of a few eggs killed in Zenker's fluid, also in corrosive-acetic-formalin. These mixtures, particularly Zenker's fluid, gave excellent results in the fertilization and early cleavage stages, but in the blastula and gastrula stages their use is not advisable since the roof of the blastocœle or gastrocœle collapses during the process of infiltration with paraffin.

Most of the observations were made on sections of eggs stained *in toto* with Grenacher's borax-carmine, differentiated in acid alcohol, and counterstained on the slide with a mixture of Lyons blue and picric acid. This treatment leaves the chromatin stained red, the yolk granules green, the centrospheres and cytoplasmic structures blue. The method has the advantage of rapidity of manipulation, an important consideration since a large number of slides are required. For the main purposes of this investigation the differentiation obtained by this method, used with care, is entirely adequate, but as a precaution against possible inaccuracy a parallel series was stained with Heidenhain's iron hæmatoxylin, which as might be expected gave better preparations, and is especially useful for the study of chromosomal vesicles.

All the figures excepting those of Plate IX. were outlined by means of a camera lucida and drawn with a magnification of about 600 diameters; in the process of reproduction they were reduced one third, and thus appear with a magnification of about 400 diameters. In all drawings of horizontal sections the orientation with reference to the plane of first cleavage is the same, and conforms to the diagrams in Plate IX.

In most cases the double nature of the cleavage nuclei appears to best advantage in horizontal sections, and might readily be overlooked were observations confined to vertical sections. It seems likely that most investigators studying the amphibian egg have confined their observations to vertical sections, since these give a better idea of the gross structure; and this circumstance leads us to suspect that conditions such as are here described for *Cryptobranchus* may occur in other amphibians and need only to be revealed by the study of horizontal sections.

Observations.

The early history of the fertilization stage of *Cryptobranchus allegheniensis* has been given in a previous paper (Smith, 1912, I.). The superficial cleavage pattern has been described in another contribution (Smith, 1912, II.).

At the time of the meeting of the germ-nuclei it is usually impossible to tell with certainty which is the egg-nucleus and which is the sperm-nucleus, but this circumstance does not affect the validity of the results recorded in this paper. The germ-nuclei do not fuse, but come to lie side by side with nuclear membranes intact (Figs. 1–4). During the long resting stage that precedes the formation of the first cleavage spindle, each germ-nucleus maintains strict individuality: there is close association, but no actual union, and certainly no mingling of structural contents. Hence, although it is convenient to employ such terms as "union of the germ-nuclei," and to speak of the "cleavage-nucleus" or "segmentation-nucleus" as if it represented a single entity, it should be understood that such expressions are loosely used, and the word "fusion" in this particular case is altogether inapplicable. It is the purpose of this paper to show that this unity or individuality of the respective germ-nuclei is not confined to the fertilization stage, but persists with great regularity during early cleavage, and with some apparent irregularities throughout the later cleavage and even into the gastrula stage.

The length of time intervening between the meeting of the germ-nuclei and the rupture of their nuclear membranes during the formation of the first cleavage spindle must be considerable, for resting germ-nuclei have been observed in eggs varying in age from twelve to twenty-three hours after fertilization. The precise time of formation of the first cleavage spindle has been determined in only one egg, which was killed twenty-six hours after artificial fertilization.

Since the germ-nuclei usually come to lie in the same horizontal plane, the most fruitful observations are obtained through the study of horizontal sections. The two asters, formed by the division of the single aster accompanying the sperm-nucleus on its journey to meet the egg-nucleus, take up positions on opposite sides of the resting germ-nuclei, in the same horizontal plane, and close to the region of contact of the germ-nuclei (Figs. 3 and 4). The initiation of the process of mitosis as evidenced by the appearance of distinct chromosomes and the rupture of the nuclear membranes does not take place in the two germ-nuclei simultaneously; one of the germ-nuclei becomes active somewhat in advance of the other (Figs. 5 and 6), thus furnishing evidence of some degree of physiological as well as structural independence.

In the fully-formed mitotic figure the two groups of chromosomes, of maternal and paternal origin respectively, remain visibly distinct (Fig. 7). This has been determined beyond a doubt in a number of cases, with no conflicting evidence. In the anaphase each of the original chromosome groups has divided equally (through the splitting of individual chromosomes), the two halves going to different poles of the spindle. Each daughter-nucleus thus receives two distinct groups of chromosomes, derived from the egg-nucleus and the sperm-nucleus respectively. Although in the anaphase and early telophase (Fig. 8) the paired groups of chromosomes are not always very sharply separated, yet there is no mingling of maternal and paternal chromosomes. Finally, in the newly-formed daughter-nucleus each parental group of chromosomes becomes metamorphosed into a separate nuclear vesicle (Figs. 9 and 10). Except for a diminution in size, which is afterwards partially made good by growth, each daughter-nucleus closely simulates the original cleavage-nucleus with its two resting germ-nuclei.

The precise stage with newly-formed vesicular daughter-nuclei has not been found in horizontal sections, hence all the figures of this stage are taken from vertical sections (Figs. 9–12). During the late phases of mitosis the two daughter-nuclei move some distance apart; their path of separation is marked by a broad trail of cytoplasm sharply defined from the surrounding yolk. The division of the centrosphere or "attraction sphere" takes place shortly before the daughter-nucleus assumes the vesicular condition. The first cleavage furrow is not yet formed, but its position, in a plane at right angles to the path of separation of the two daughter-nuclei and midway between them, is visibly indicated by a condensation of cytoplasm in the blastodisc.

Aside from the fact that the two nuclear vesicles of a newly-formed daughter-nucleus are always situated approximately equidistant from the plane of first cleavage, the orientation of these nuclear vesicles with reference to each other is decidedly variable. Since the two original germ-nuclei at the time of meeting usually lie in the same horizontal plane (Figs. 2–4), we should expect the same thing of the two vesicles of a daughter-nucleus; but in the preparations studied these were more often found to lie in a plane oblique to the horizontal, and sometimes one vesicle lies directly above the other (Figs. 9 and 10). It should be remembered, however, that the original germ-nuclei do not always lie in the same horizontal plane (Fig. 1). Moreover, the stage with newly-formed daughter-nuclei was not represented in horizontal sections, which are naturally most favorable for the discovery of nuclei consisting of vesicles lying in the same horizontal plane. There is no external landmark by means of which this stage may be recognized, and it is passed through very rapidly; in the making of horizontal sections the available material was exhausted without finding the precise stage desired.

In the study of the subsequent behavior of the nuclei the early cleavage furrows serve as convenient landmarks for orientation, and it is necessary to keep in mind the elementary fact that the second cleavage furrow forms at right angles to the first, and that a similar alternation in the direction of successive cleavage furrows is characteristic of the entire process of cleavage. Of more immediate importance to us is the correlated fact that the second nuclear division occurs in a direction at right angles to the first nuclear division, and that this alternation in the direction of successive nuclear divisions is repeated throughout cleavage. To be sure, this is strictly true only in the early cleavage stages, for there is increasing irregularity in the direction of both cleavage furrows and nuclear divisions throughout the later stages of cleavage. Incidentally, it should be noted that nuclear division always precedes the formation of the corresponding cleavage furrow.

In preparation for the second nuclear division, the two asters of a single daughter-nucleus take up positions on opposite sides of the nucleus, in the same horizontal plane and with a line connecting them parallel to the first cleavage furrow which is now usually in process of formation (Figs. 12–20; 23–26). In case the two nuclear vesicles of a single daughter-nucleus are not already ranged midway between the two asters, they rotate to this position (compare Figs. 15–16 with Figs. 17–18). Typically, the two nuclear vesicles come to lie in the same horizontal plane (Figs. 17–20, 23–26); but this is not the invariable position, for in three different eggs they were found to lie one above the other, as exemplified in Figs. 21 and 22. In any case the final position assumed insures an equal division of maternal and paternal chromatin-groups in the ensuing mitosis. Thus the second cleavage mitosis (Figs. 27 and 28) is in all essential respects a repetition of the first, though the direction of the spindle is different. Throughout the entire segmentation period there is nothing in the behavior of the nuclei more impressive than the precision and regularity with which the two nuclear vesicles of a single nucleus are ranged side by side in the plane of the equatorial plate in preparation for the formation of the spindle.

In the resting stage following the second mitosis the two nuclear vesicles of each nucleus usually lie in the same horizontal plane (Figs. 29, 30, 32); their entire orientation indicates their derivation from the two groups of chromosomes at one pole of the spindle in the anaphase and early telophase of the preceding mitosis (Fig. 28). Occasionally, though not so often as in the preceding resting stage, one nuclear vesicle is found situated above the other (Fig. 31).

In *Cryptobranchus* the third cleavage furrows are vertical and intersect the second furrows at some distance from the first (Plate IX., Fig. 45, *J*). Thus the third cleavage furrows are nearly parallel to the first, but diverge slightly from it.

In preparation for the third cleavage mitosis the two asters take up positions on opposite sides of the nucleus, in such a situation that a line connecting them is nearly parallel to the plane of second cleavage (Figs. 33, 34; Plate IX., Fig. 45, *H*). In all cases the two nuclear vesicles entering the mitotic figure are ranged equidistant from the two asters (Figs. 33, 34, 35), and in a slightly earlier stage may be found rotating to this position (Fig. 32). Thus in the third cleavage mitosis (Fig. 36) the segregation and equal division of maternal and paternal chromosome groups is maintained. The direction of the axis of the spindle is at right angles to the preceding mitosis, and parallel to the first (Plate IX., Fig. 45, *H* and *I*). Upon the completion of mitosis the third cleavage furrows form as described in the preceding paragraph. At this time the four nuclei on the same side of the second cleavage furrow sometimes lie precisely in the same vertical plane, so that they are all visible in a single section taken parallel to the second cleavage furrow.

The fourth cleavage furrows combine to form a circular, or more often oval, figure with its longer axis coinciding with the plane of second cleavage. This circular cleavage divides the egg very unequally, cutting off eight micromeres from eight comparatively large macromeres. Preparation for this cleavage involves rotation of the two nuclear vesicles of a single daughter-nucleus of the third cell division from their original position as shown in Fig. 37 to the position shown in Fig. 38. This latter orientation insures the maintenance of the segregation of the germ-nuclei in the fourth mitosis (Figs. 39–42). As in the previous divisions, the maternal and paternal chromosome groups have here been traced through the various phases of mitosis to the late anaphase and early telophase, where they are still distinctly separate. The position of the vesicles of the newly-formed daughter-nucleus (Figs. 43 and 44) indicates that one is of maternal origin, the other paternal. The results as regards the orientation of the nuclei up to this point are summarized in the diagrams of Plate IX., Fig. 45.

In the later cleavage divisions there is increasing irregularity in the direction of spindle axes and cleavage furrows, but this does not constitute any serious obstacle to tracing the history of the germ-nuclei since the really important point is the orientation of the nuclear vesicles with respect to the axis of the spindle. As already indicated, this is invariably such as to insure the segregation and equal representation of maternal and paternal chromosome groups in the daughter-nuclei. As the nuclei become smaller, it sometimes is difficult to distinguish the two groups of chromosomes in the late phases of mitosis, but comparison with slightly earlier and later phases leaves no doubt as to the continuity of the respective germ-nuclei. Until an advanced cleavage stage, the double structure of the resting nuclei is almost always clearly demonstrable. As the nuclei become more numerous, it becomes easier to find nuclei cut in such a manner as to show distinctly the double structure. In the more active regions of the egg, the synchronism of division of the different cells is maintained to the late blastula stage, so that there is little likelihood of confusing cell-generations. Between micromeres and macromeres there is a fine gradation in phases of the nuclear cycle, so that a close series may be readily obtained. The individuality of the germ-nuclei has been traced without a break to an advanced cleavage stage, and it would not be difficult to illustrate this entire result with an adequate series of figures, but such a procedure would require an enormous amount of time and unduly extend the limits of this paper.

Beginning with about the seventh cell generation certain irregularities, real or apparent, occur with increasing frequency to mar the mechanical precision of the events thus far described in the history of the germ-nuclei. In the resting stage the two nuclear vesicles of a single nucleus are often deeply lobed, sometimes in such a manner as to give the impression that the nucleus is made up of many vesicles. In the late telophases the nucleus is often really made up of several or many separate though closely aggregated vesicles, but this is undoubtedly a part of the usual process of mitosis, as a more searching examination of these late phases throughout cleavage will readily show.

The transformation of the daughter-nucleus into the vesicular condition must take place with considerable rapidity, especially in the early cleavage stages, for some phases of this process have not been observed earlier than the fourth mitosis, and only occasionally in the fourth and fifth mitoses. In more advanced cleavage there is little difficulty in finding material representing the entire history of the telophase.

In the early anaphase, the long thread-like chromosomes are V-shaped, with the limbs of the V almost parallel and very straight; the apex is directed away from the equatorial plate. In a slightly later phase, which we may call the late anaphase, the chromosomes are still roughly V-shaped but finely undulating except at their free ends. A little later (early telophase) each chromosome becomes much convoluted, often coiled in a loose irregular spiral; this change begins at the apex and proceeds toward the free ends. The next step consists of the metamorphosis of each chromosome into a chromosomal vesicle, a process which I have been able to follow in considerable detail, but will not attempt to describe here. About this time the centrosphere or "attraction sphere" divides.

During the anaphase and early telophase the maternal and paternal chromosome groups are almost always clearly separated, sometimes with a broad space between the two groups. The segregation of the chromosomal vesicles into two groups, maternal and paternal respectively, is usually evident. In the late telophase the chromosomal vesicles of each group unite to form separate nuclear vesicles which for a time show indications of their manner of origin through the persistence of lobes separated by deep clefts. The inner boundaries of the chromosomal vesicles persist for a time as partitions in the nuclear vesicles, but finally disappear wholly or in part. In the late blastula the vesicular nucleus in many cases retains a lobed structure throughout the resting stage. It is possible that in some cases the fusion of chromosomal vesicles to form vesicular nuclei of the usual duplex type is never completed, for in the late blastula and early gastrula nuclei consisting of three, four or even more well-rounded vesicles are not uncommon.

How much longer and to what extent during the development of the embryo a strict separation of the germ-nuclei is maintained I have not attempted to determine, but it is not necessary to conclude that it continues throughout the life of the organism, either in the somatic or the germ cells. In the discussion it will be shown that the individuality of the germ-nuclei is not incompatible with a mingling of maternal and paternal chromosomes, and there is no biological necessity for a strict separation of the germ-nuclei in any stage from fertilization to synapsis.

Discussion.

In 1875 Oscar Hertwig and Hermann Fol showed that the fertilized egg contains two nuclei, one belonging to the egg itself and the other introduced by the spermatozoön. While the earliest observers of the process of fertilization, notably Auerbach, Strasburger and Hertwig, described the complete fusion of these germ-nuclei to form the first embryonic nucleus, called by

Hertwig the cleavage-nucleus or segmentation-nucleus, later observations showed that such is not always the case. In 1881 Mark demonstrated that in the slug *Limax* the two germ-nuclei do not fuse; after coming together they persist during the formation of the two cleavage centers, then their membranes gradually disappear. Two years later Van Beneden showed that in *Ascaris*, not only do the germ-nuclei become apposed without fusion, but each gives rise to an independent group of chromosomes which contribute equally to the formation of the daughter-nuclei. Thus the foundation of the doctrine of the biparental character of the nuclei of sexually-produced organisms was laid down by Van Beneden. In many animals, and in some plants (gymnosperms), the independence of the formation of the maternal and the paternal chromosome groups following fertilization has been established by direct observation, though the demonstration has seldom been carried beyond the first cleavage stage. On the other hand there are some animals, and many plants, in which the germ-nuclei meet and fuse while in the resting condition, so that in the chromatin of the resulting nucleus maternal and paternal contributions cannot be readily distinguished.

Häcker (1892 and 1895) and Rückert (1895) found that the germ-nuclei of *Cyclops* do not fuse but preserve their individuality throughout at least a considerable period of the cleavage of the egg. In mitosis the two groups of chromosomes, of maternal and paternal origin respectively, remain distinct and bilaterally distributed, while each resultant daughter-nucleus in the resting stage consists of two closely apposed but structurally separate vesicles.

In 1901 Conklin described the double structure of the cleavage nuclei of *Crepidula* in certain stages of the nuclear cycle, and pointed out that the two halves which at times appear as distinct entities are almost certainly to be regarded as of maternal and paternal origin respectively. "This separateness is most easily observed in the telophase of each division, though in some cleavage cells it may be seen in the prophase also, or even throughout the resting period. At the time when the daughter-nuclei are being formed the chromosomal vesicles fuse into two groups which are closely pressed together but still separated by a partition wall, as Rückert has shown to be the case in *Cyclops*. Gradually this partition wall disappears, being preserved longest on that side of the nucleus nearest the centrosome. Here a groove is formed on one side of the nucleus which marks the line of contact between the two halves. In some cleavage cells this groove is visible throughout most of the resting period; in others it disappears during the greater part of the resting period, though it may reappear in the following prophase; in all cases, however, the partition wall and groove reappear in the next succeeding telophase, when it is again formed in the manner described above. I have observed the double character of the nucleus in the telophase of every cleavage up to the 29-cell stage, and in several of the later cleavages up to the 60-cell stage, though it becomes more difficult to see as the nuclei grow smaller. . . . It still remains to show that these double nuclei really represent the egg and sperm nuclei which have not yet lost their individuality. This cannot be demonstrated in *Crepidula*, for the reason that this double character is not apparent at every stage in the nuclear cycle, but it is extremely probable" (Conklin, 1901). Additional observations recorded by Conklin in support of his interpretation may be summarized as follows: (1) In the metaphase of the first cleavage division the maternal and paternal germ-nuclei are represented by separate groups of chromosomes; in the early anaphase these groups of chromosomes can no longer be distinguished, but the nuclei are clearly double in the immediately following late anaphase and telophase, and the position of the partition wall in these double nuclei corresponds to the plane of contact between the germ-nuclei. (2) The groove which is found on one side of the nucleus in the telophase of the first cleavage mitosis persists well into the resting stage, and a corresponding groove is found in the same position in the prophase of the second mitosis. The central spindle for the second cleavage mitosis lies in this groove, and thus the amphiaster actually lies in the only plane in which it would be possible to halve the two parts of the double nuclei. Although cleavage divisions successively alternate in direction, unequal division of the double nuclei is prevented either by a rotation of the nucleus during the resting stage, or by a rotation of the spindle in the early stage of mitosis. (3) In certain abnormal cleavages the double nuclei are really two entirely separate nuclei lying side by side within a single cell. (4) In each of the germ-nuclei, before they come into contact, there is a single nucleolus; these nucleoli disappear in the prophase of the first cleavage, but in the succeeding telophase a single nucleolus generally appears in each half of each daughter-nucleus. The same is true of the succeeding cleavages, so that each nucleus throughout the cleavage usually has two nucleoli in the telophase or early resting stage.

Beard (1902) described a double structure of the resting stages of the nuclei of the early germ cells of *Raja batis;* these were not traced earlier than a late gastrula stage, but influenced by the findings of Häcker and Rückert, Beard did not hesitate to interpret the double nuclei as consisting of distinct maternal and paternal halves.

Jenkinson (1904) gives some interesting figures of the fertilization and first cleavage stages of *Axolotl.* The germ nuclei meet without fusion, and the chromosomes appear separately in each pronucleus while the nuclear membranes are still intact. In some cases at least, these two chromosome groups remain distinct in the equatorial plate after the dissolution of the nuclear membranes. Scant attention is paid to these features in the text of Jenkinson's paper, which is concerned with other matters, but the author states that he has found two distinct sets of chromosomes in some preparations of the fertilization spindle of *Triton.*

In 1904 Moenckhaus described the independence of the maternal and paternal chromosome groups in the early cleavage spindles of the hybrids produced by fertilizing the eggs of *Fundulus* with *Menidia* sperm. The difference in the size and shape of the chromosomes of the two species makes the identification of the maternal and paternal chromosomes in the case described a matter of certainty.

Pinney (1918) found that two nucleoli are typically present in the nuclei of normal *Ctenolabrus* blastoderms, and cited evidence supporting the view that in these double nucleoli observed in fishes we are dealing with parental homologues.

Concerning *Cryptobranchus alleghenienses*, the writer believes that the observations recorded in the present paper establish beyond question the complete separation of maternal and paternal germ-nuclei to a late blastula stage at least. The separation is particularly marked during the resting stage of the nuclear cycle, precisely where most investigators working with other species have encountered the greatest difficulty.

The observations thus far cited indicate that in certain forms the individuality of the germ-nuclei during early embryonic development is maintained by complete separation of the nuclear material derived from the egg and the spermatozoön respectively. It has already been indicated that this segregation is not by any means a universal phenomenon. Is it possible that the individuality of the germ-nuclei may be maintained, in all essential respects, in those other cases where there is a mingling of chromatin derived from the two germ-nuclei? Let us first examine the facts that require explanation.

In all cases where the germ-nuclei fuse into a single vesicular nucleus before the formation of the first cleavage spindle, mingling of maternal and paternal chromosomes may be expected. Sax (1918) has recently described two cases in flowering plants, *Fritillaria* and *Triticum*. In *Fritillaria* the germ-nuclei usually unite while in the resting condition, although occasionally they are in the spireme stage at the time of fusion. The presence of a single spireme in the zygote could not be demonstrated. In *Triticum* the sperm-nucleus is small and almost homogeneous in structure even while in contact with the egg nucleus. The sperm-nucleus enters the egg-nucleus and there forms a separate compact spireme; at the same time the spireme of the egg-nucleus is formed. In both *Fritillaria* and *Triticum* the maternal and paternal chromosomes are formed independently, but they are not found in separate groups.

Moenckhaus (1904) found that after the first few cleavage divisions in his hybrid teleost eggs the chromosomes of maternal and paternal origin mingled indiscriminately upon the equatorial plate, and the observations of Morris (1914) are in agreement.

Metz (1916) has found in the diptera a pairing of homologous chromosomes, simulating synapsis, which occurs in all tissues, somatic as well as germinal; this association of maternal with paternal chromosomes was found in late cleavage and during all later stages of embryonic development. From the following statement by Overton (1909) we have a suggestion of a similar occurrence in plants, though attention is directed more particularly to the matter of genetic continuity of individual chromosomes: "In the somatic nuclei (of certain plants) the chromosomes are represented during rest by definite visible bodies, the pro-chromosomes, which are arranged in parallel pairs, with apparent linin intervals. These heterogeneous spirems, the homologous portions of which have early become associated in pairs, probably remain distinct throughout the life-history of the sporophyte."

In spite of the mingling and even paired association of maternal and paternal chromosomes, there are reasons for believing that the two kinds of chromosomes maintain their independence until gametogenesis. This leads us to a consideration of the doctrine of the genetic continuity of individual chromosomes, which goes further than the principle of duality of the embryonic nuclei, but confirms it as a universal law.

The remarkable constancy in the number of chromosomes throughout the cells of a given organism and species has long been known, and affords important evidence for the view that the chromosomes are persistent as individual structures. To be sure, it sometimes happens in mitosis that one or more chromosomes belonging to one daughter-group, accidentally become included with the other group so that one of the daughter-nuclei has fewer, the other more, than the normal somatic number; but such an occurrence is very exceptional, and in subsequent divisions of these cells the number of chromosomes appearing is not the normal, but the increased or diminished number (Boveri, 1890). Whatever the number of chromosomes entering into the formation of a resting nucleus, the same number afterwards issues from it.

In 1883 Van Beneden showed that in *Ascaris* the spermatozoön brings in just as many chromosomes as are contained in the egg. As a result of a careful study of mitosis in epithelial cells of the salamander, Rabl (1885) concluded that the chromosomes do not lose their individuality at the close of division, but persist in the chromatic reticulum of the resting nucleus. Boveri (1887 and 1888) supported Rabl's hypothesis on the ground of his own studies and those of Van Beneden on the early stages of *Ascaris*. Boveri demonstrated in *Ascaris* that in the formation of the spireme the chromosomes reappear in the same position as those which entered into the formation of the reticulum, precisely as Rabl had maintained. As the long chromosomes diverge, their free ends are always turned toward the equator of the spindle, and upon the reconstruction of the daughter-nuclei these ends give rise to corresponding lobes of the nucleus, which persist throughout the resting stage. At the succeeding division the chromosomes reappear exactly in the same position, their ends lying in the nuclear lobes as before. These observations were afterwards confirmed by Herla (1893), and more recently Sutton (1902) has observed practically the same thing in *Brachystola magna*. Boveri (1891) concluded that the chromosomes must be regarded as individuals that have an independent existence in the cell, and expressed his belief that "we may identify every chromatic element arising from a resting nucleus with a definite element that entered into the formation of that nucleus, from which the remarkable conclusion follows that in all cells derived in the regular course of division from the fertilized egg, one half of the chromosomes are of strictly paternal origin, the other half of maternal."

Herla (1893) and Zoja (1895) have shown that if in *Ascaris megalocephala* the egg of variety *bivalens*, having two chromosomes, be fertilized with the spermatozoön of variety *univalens*, having one chromosome, the three chromosomes reappear at each cleavage, at least as far as the twelve-cell stage; and according to Zoja, the paternal chromosome is distinguishable from the two maternal at each step by its smaller size. "We have thus what must be reckoned as more than a possibility, that every cell of the body of the child may receive from each parent not only half of its chromatin substance, but one half of its chromosomes, as distinct and individual descendants of those of its parents" (Wilson, 1900).

Boveri (1909) found in *Ascaris* that in sister cells preparing for division the configuration of the groups of chromosomes is the same. The similarity of the sister cells is explicable on the view that the chromosomes retain during the resting stage the same shape and size and relative location that they had at the end of the preceding division. In cells of these same embryos that are not sister cells, a great variety of arrangements of the chromosomes is found, and no two arrangements are so nearly alike as are those found in sister cells.

Other evidence for the continuity of individual chromosomes is derived from those cases where the reconstruction of the resting nucleus takes place through the metamorphosis of each chromosome into a hollow vesicle, and the aggregation or fusion of these chromosomal vesicles to form a single nucleus. Such, to be sure, is not the only type of telophase (Wilson, 1900, p. 71), but it takes place in many segmenting ova and in some spermatogonia. According to Conklin (1902), in the late stages of mitosis of the segmenting egg of *Crepidula* the chromosomes enlarge to form vesicles and these unite into a resting nucleus; the nuclear membrane is composed of the outer walls of the vesicles, while the inner walls stretch through the nucleus as chromatic partitions; the chromosomal vesicles from the egg and sperm nuclei respectively remain distinct longer than those from the same germ nucleus. Vesicular chromosomes have been described in fish eggs by Moenckhaus; the individual vesicles fuse with their neighbors and these larger ones with each other until at last the entire nucleus is simply one great vesicle, which is at first lobed, but later is well rounded. Wenrich (1916) says of *Phrynotettix*: "The spermatogonial divisions showed that each chromosome forms a sac or vesicle in the earlier telophases, and that it expands and becomes diffused within these vesicles; that, although the vesicles appeared to coalesce, there is always a remnant of each chromosome visible in the center of the region occupied by the vesicle, and that in the prophase the chromatin concentrates about this remnant or core and there forms a spirally coiled thread, which develops into a prophase chromosome." Richards (1917) has shown that in the cleavage and gastrula stages of *Fundulus* and *Coregonus* the chromosomes in the telophase of mitosis enlarge to form vesicles which remain distinct though compactly massed together during the resting stage. The new chromosome arises within a vesicle, through the aggregation of its granules; thus there is genetic continuity of individual chromosomes. According to Richards, the polarity of the cell is manifested in the arrangement of the elongated vesicles. When the centrosome divides, the cell acquires a new axis at right angles to the old, in a line connecting the two asters; the chromosomes, when formed, orient themselves with respect to the new axis. Pinney (1918) figures chromosomal vesicles in the telophase of dividing blastomeres of *Ctenolabrus* × *Fundulus* hybrids. Some observations on chromosomal vesicles in *Cryptobranchus alleghenensis* are included in the present paper. I have examined a few sections of *Coregonus* blastoderms belonging to the collection of the Michigan State Normal College and the material appears to be exceptionally favorable for the study of chromosomal vesicles.

Thus it has been proved that in the fertilized egg one half of the chromosomes are derived from the father and one half from the mother, and that at every division of the egg the chromosomes also divide in such a manner that their progeny are distributed in equal number to all the cells of the egg. Further, there is genetic continuity between each individual chromosome that enters the resting stage and a corresponding chromosome that emerges in preparation for the next division. The conclusion is thus reached that the fertilized egg, and all the cells derived from it, contain a double set of chromosomes, paternal and maternal. This conclusion implies that at every step the respective chromosome groups preserve their independence, however much they are mingled with one another.

This conclusion is further strengthened by observations of individual differences, both morphological and physiological, between the chromosomes of a given simplex group, and by the behavior of the chromosomes in synapsis. The chromosomes of a single group, maternal or paternal, are not precisely alike, but differ among themselves in size, form and genetic potency (Montgomery, 1901; Sutton, 1902 and 1903; Morgan, 1915). In certain species the size differences are very marked, so that the chromosomes of a single germ-nucleus may be arranged in a graded series; these size differences are constant from one cell-generation to the next, so that individual chromosomes may be identified in successive cell-divisions. In other species where the visible differences are not so marked we have evidence that physiological differences exist, for Boveri (1907) has shown the strong probability that normal development of the egg is possible only in the presence of at least a single set of qualitatively different chromosomes. Thus the concept of the individuality of the chromosomes has been extended to include not merely the genetic continuity of each particular chromosome, but also its idiosyncrasy or specificity.

In any biparental organism, the duplex chromosome group is composed of two equivalent parental series or simplex groups, in which each individual chromosome is homologous with a very similar chromosome belonging to the other series; in other words, the chromosomes are present in biparental pairs (Montgomery, 1901; Sutton, 1902 and 1903; Wilson, 1912). "In the process known as synapsis, which takes place shortly before the last two cell-divisions concerned in the formation of the germ cells, the chromosomes do in fact unite in pairs, two by two. There is reason to believe that the two members of each pair are respectively of maternal and paternal derivation; and the probability of this view, first stated by Montgomery, has steadily increased." (Wilson, 1913). In one of these two final divisions of the germ cell cycle the double chromosome groups are reduced to single ones in preparation for the subsequent process of fertilization; this reduction is accomplished through the failure of the individual chromosomes to split in the process of mitosis. In this reducing division the two members of a synaptic pair are separated to pass into different daughter cells, but not necessarily in such a manner that each daughter cell receives exclusively maternal or exclusively paternal chromosomes. The distribution of each pair of homologous chromosomes is entirely independent of that of every other pair, so that in the daughter cells combinations of non-homologous chromosomes occur regardless of their parental origin; each daughter cell usually receives a mixture of maternal and paternal chromosomes in varying proportions. Within the limits of the reduced number, any combination of the chromosomes furnished by the immature germ cell is possible in a daughter cell resulting from the reducing division, save that a single daughter cell cannot receive both members of a synaptic pair. Synapsis is thus explained by the provision which it makes that two homologous chromosomes shall in no case enter the nucleus of a single spermatozoon or mature egg (Sutton, 1902). The principle of random distribution or independent assortment of non-homologous chromosomes has been confirmed by the direct observations of Carothers (1913 and 1917), Wenrich (1915 and 1916) and others. The point that immediately concerns us here is the fact that in the reducing division the individuality of the germ-nuclei is usually lost, for it seldom happens that a single daughter cell, and consequently a single mature gamete, receives exclusively maternal or exclusively paternal chromosomes.

"Synapsis is not a haphazard junction of chromosomes, but an orderly union of elements of maternal and paternal origin, similar in size, in details of form, and probably also in function" (Kellicott, 1913, p. 294). The orderly processes of heredity as they exist today in biparental organisms would be impossible were the integrity of the maternal and paternal chromosome groups not maintained up to the time of synapsis. In *Cryptobranchus allegheniensis* and in some other forms this integrity is manifested at every step by a complete segregation of maternal and paternal chromosome *groups;* in other forms the independence of the chromosome groups is maintained in spite of the mingling of maternal and paternal chromosomes. The persistent individuality of the chromosomes is the important thing, and so long as this is maintained the germ-nuclei exist as actual entities whether visibly separated or not. The distinction between the two classes of germ-nuclei—those that fuse at the time of fertilization and those that do not—is more apparent than real. The expression "individuality of the germ-nuclei" seems justified in either case, for individuality implies separableness as well as separateness (Conklin, 1916); but some may prefer to use the term "autonomy," which places the emphasis upon independence. Since attention has become focused upon the chromosomes, the expression "autonomy of the maternal and paternal chromosome groups" has often been used to indicate the duality of nuclear structure.

Where it exists, the separation of maternal and paternal chromatin-complexes into distinct groups within a single nucleus affords a striking exemplification of the deeper and more universal truth that each germ-nucleus is represented in its entirety in every cell, somatic as well as germinal, of a developing organism. In *Cryptobranchus alleghenіensis* this dual structure of the nucleus is clearly visible in every cell of the early segmentation stages at least, so that here we have material for an ocular demonstration of a principle long ago foreseen by Huxley (1878), who wrote: "It is conceivable, and indeed probable, that every part of the adult contains molecules derived from both the male and the female parent; and that, regarded as a mass of molecules, the entire organism may be compared to a web of which the warp is derived from the female and the woof from the male."

SUMMARY.

In the fertilization of the egg of *Cryptobranchus alleghenіensis* the germ-nuclei do not fuse, and in the first cleavage mitosis each gives rise to a separate group of chromosomes whose descendants pass separately to the daughter-nuclei.

During the ensuing resting stage each germ-nucleus is represented by a structurally distinct vesicle. The separateness of the germ-nuclei is thus maintained throughout the entire nuclear cycle.

Throughout early cleavage the nuclear divisions are of the same duplex type, and the resting nuclei are always distinctly

double. The genetic continuity of each half of the double nucleus has been clearly traced to an advanced cleavage stage.

During late cleavage and in the early gastrula the nuclei are still typically double, but certain irregularities which tend to disguise the double structure occur with increasing frequency and the segregation of maternal and paternal chromatin cannot always be demonstrated.

The hypothesis of individuality of the germ-nuclei as applied to those species in which there is a mingling of maternal and paternal chromosomes is discussed, and supported by considerations regarding the persistent individuality of the chromosomes.

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Explanation of Plate I.

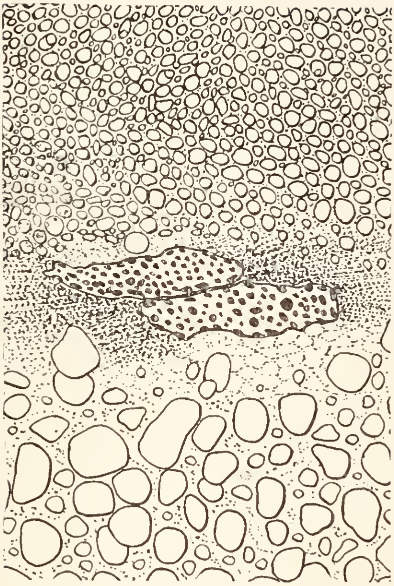
Cryptobranchus alleghenensis.

Fig. 1. Portion of a meridional section of a fertilized egg, showing the meeting of the sperm-nucleus and the egg-nucleus. Each germ-nucleus extends through four sections, of which an inner one is shown in the figure. The section passes midway between the two asters, which lie on opposite sides of the nuclei in a line at right angles to the plane of the section. The egg was fixed with bichromate-acetic formalin and stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 2. Meridional section of an egg killed twenty-two and one half hours after fertilization, showing the resting germ-nuclei. Each germ-nucleus extends through five sections, of which the middle one is here shown. The section passes midway between the two asters, which lie close to the apposed surfaces of the two nuclei in a line oblique to the plane of the section. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 3. Horizontal section through the resting germ-nuclei of an egg killed twenty-one and one half hours after fertilization. Nearly half of each nucleus is left in an adjacent section. Two asters are shown in the figure. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 4. Horizontal section through the resting germ-nuclei of an egg killed twenty-two and three fourths hours after fertilization. Each germ-nucleus extends through three sections, of which the middle one is here shown. Two asters are shown in the figure. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.



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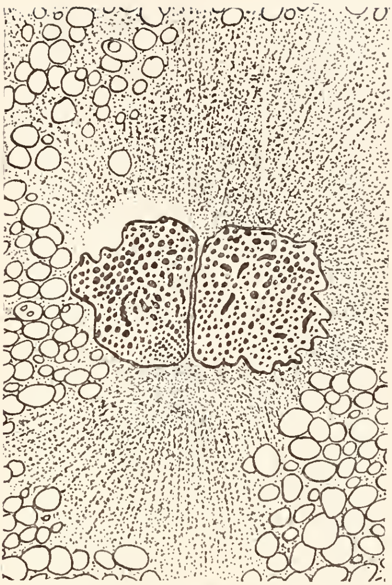

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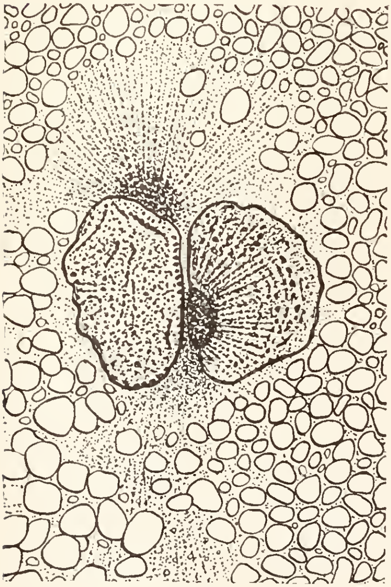

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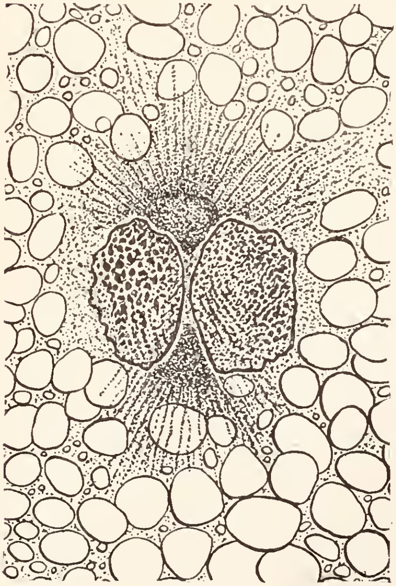

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EXPLANATION OF PLATE II

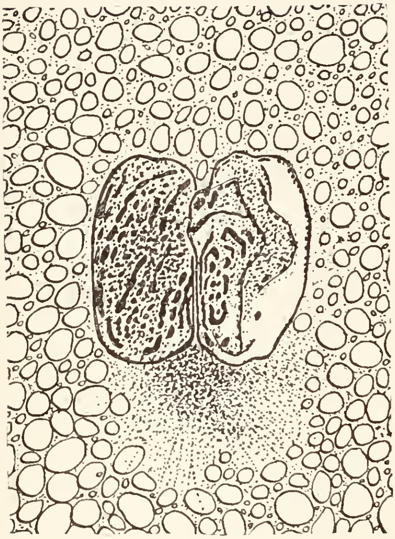
Cryptobranchus allegheniensis.

FIG. 5. Horizontal section of a fertilized egg showing the germ-nuclei preparing for the first cleavage mitosis. The germ-nucleus shown at the right is in a slightly more advanced phase than the other. A portion of each nucleus is left in an adjacent section. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

FIG. 6. Horizontal section of an egg killed twenty-two and one half hours after fertilization, showing the germ-nuclei preparing for the first cleavage mitosis. The germ-nucleus shown at the right is in a more advanced phase than the other. About half of each nucleus is left in an adjacent section. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

FIG. 7. Horizontal section through the first cleavage spindle (late prophase). The chromosomes are segregated into two groups, of maternal and paternal origin respectively. A very small portion of each group of chromosomes is left in each of the adjacent sections. Bichromate-acetic-formalin fixation; stained with iron-hæmatoxylin. × 400.

FIG. 8. Horizontal section of an egg killed twenty-six hours after fertilization, showing a late stage (early telophase) of the first nuclear division. In the distribution of chromatin to the daughter-nuclei, the segregation of maternal and paternal chromatin is maintained. A very small portion of the chromatin of each daughter-nucleus is left in an adjacent section. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric acid. × 400.



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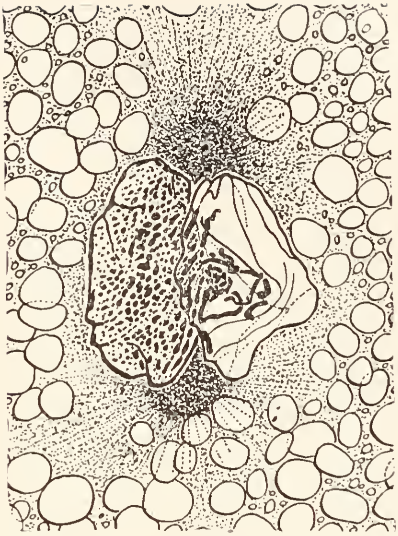

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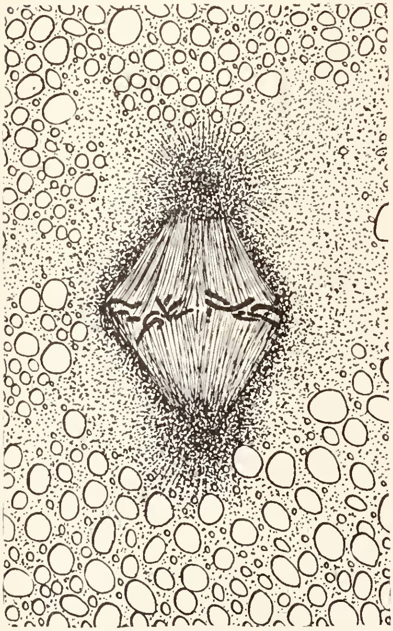

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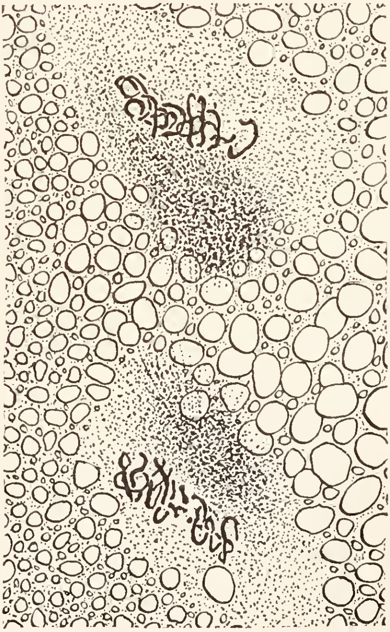

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Explanation of Plate III.

Cryptobranchus alleghenіensis.

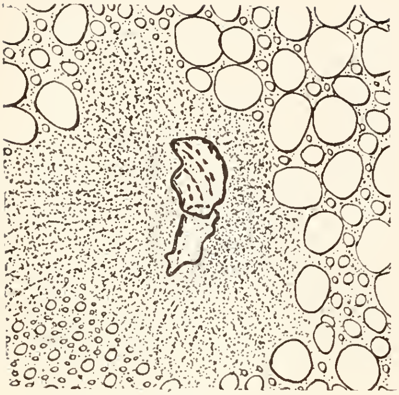
Figs. 9 and 10. Two successive vertical sections through a single daughter-nucleus of the first cleavage mitosis. The sections are nearly meridional, and pass very close to the other daughter-nucleus, hence at right angles to the plane of the first cleavage furrow which is not yet formed. The nucleus shown is almost entirely confined to the two sections. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric acid. × 400.

Fig. 11. Vertical section through a single daughter-nucleus of the first cleavage mitosis. The section is nearly meridional, and passes very close to the other daughter-nucleus, hence at right angles to the plane of the first cleavage furrow which is not yet formed. A considerable part of each nuclear vesicle is left in an adjacent section, and comparison with these other portions shows that the plane of contact of the two nuclear vesicles is oblique to the plane of the section. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 12. Vertical section showing a single daughter-nucleus of the first cleavage mitosis, taken at right angles to the plane of the first cleavage furrow which is not yet formed. The entire double nucleus is confined to this section. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 13. Vertical section, parallel to the newly-formed first cleavage furrow, through a single daughter-nucleus of the first cleavage division. Part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 14. Vertical section, parallel to the first cleavage furrow, through the other daughter-nucleus of the egg used for Fig. 13. Part of the nucleus is left in an adjacent section, where the two nuclear vesicles are more closely united. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.



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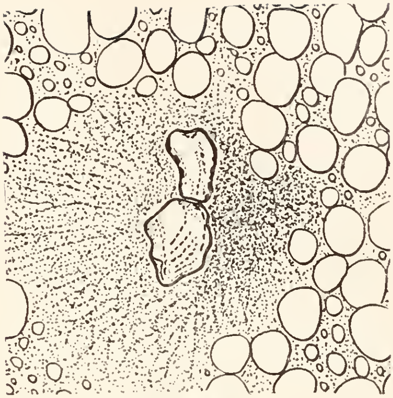

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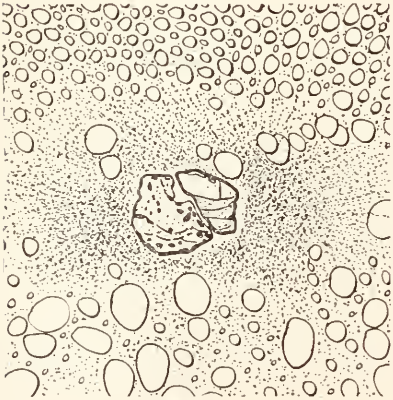

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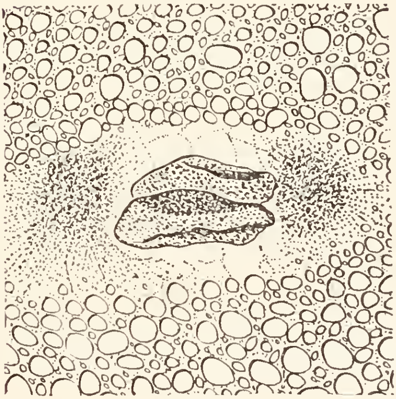

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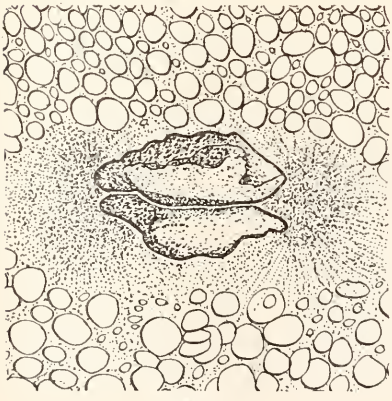

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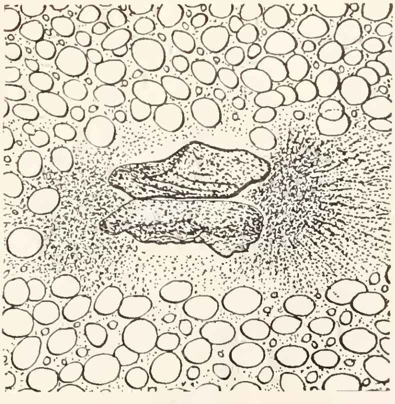

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Explanation of Plate IV.

Cryptobranchus alleghenienis.

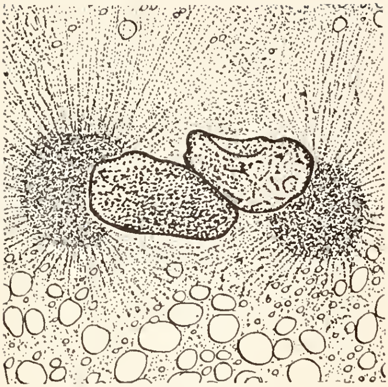
Figs. 15 and 16. Two successive horizontal sections showing a single nucleus rotating into position for the second cleavage mitosis; almost the entire nucleus is confined to these two sections. The newly-formed first cleavage furrow extends in a direction parallel to a line connecting the two asters. Fixed in bichromate-acetic-formalin; stained with iron-hæmatoxylin. × 400.

Fig. 17. Horizontal section through a single nucleus in position for the second cleavage mitosis; the greater part of the nucleus lies in this section. The newly-formed first cleavage furrow extends in a direction parallel to a line connecting the two asters. Fixed in Zenker's fluid; stained with iron-hæmatoxylin. × 400.

Fig. 18. Horizontal section through the other nucleus of the egg containing the nucleus shown in Fig. 17. Orientation as in the preceding figure. Stained with iron-hæmatoxylin. × 400.

Fig. 19. Horizontal section showing a single nucleus in position for the second cleavage mitosis; the entire nucleus lies in this section. The newly-formed first cleavage furrow extends in a direction parallel to a line connecting the two asters. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 20. Horizontal section through the other nucleus of the egg used for Fig. 19. A small part of one nuclear vesicle, and the greater part of the other, lies in an adjacent section. Orientation as in the preceding figure. Stained with borax-carmine Lyons-blue picric-acid. × 400.



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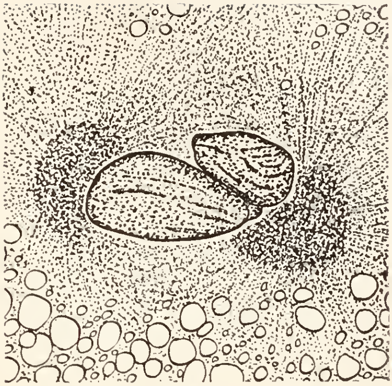

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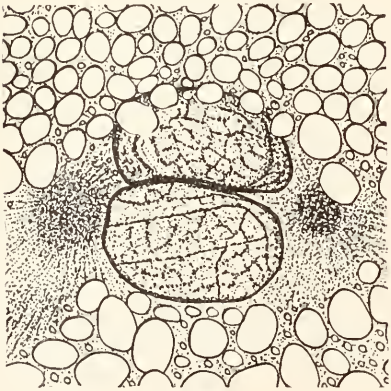

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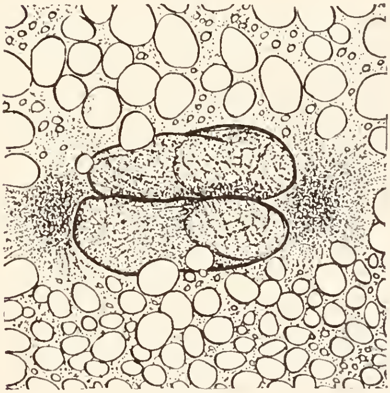

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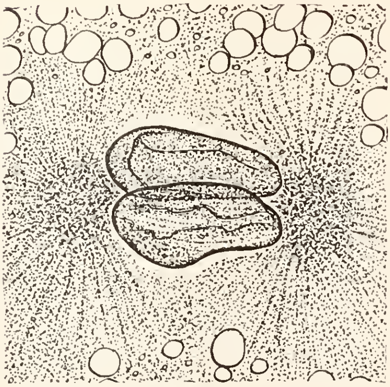

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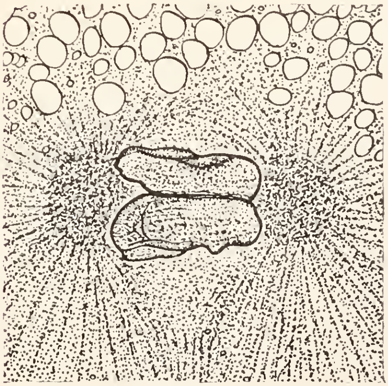

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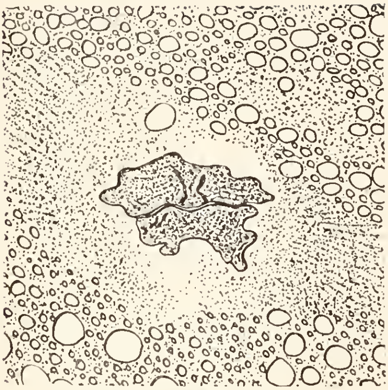
EXPLANATION OF PLATE V.

Cryptobranchus allegheniensis.

FIGS. 21 AND 22. Two successive meridional sections through a single nucleus preparing for the second cleavage mitosis. The sections extend at right angles to the first cleavage furrow, and close to the other cleavage-nucleus. A considerable part of the nucleus here shown is left in an adjacent section where it is not so clearly separated into two nuclear vesicles. Fixed in bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

FIG. 23. Horizontal section through a single nucleus preparing for the second cleavage mitosis; the nucleus is almost entirely confined to this section. The first cleavage furrow extends in a direction nearly parallel to a line connecting the two asters. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

FIGS. 24, 25 AND 26. Three successive horizontal sections through a single nucleus entering upon the second cleavage mitosis; all but a very small portion of the nucleus is included in these sections. The first cleavage furrow extends in a direction nearly parallel to a line connecting the two asters. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.



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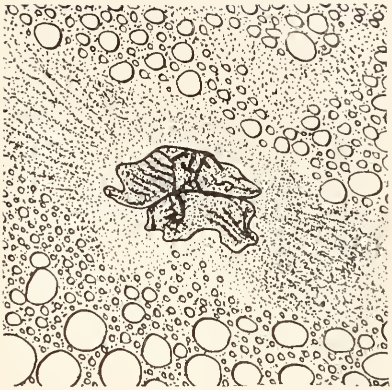

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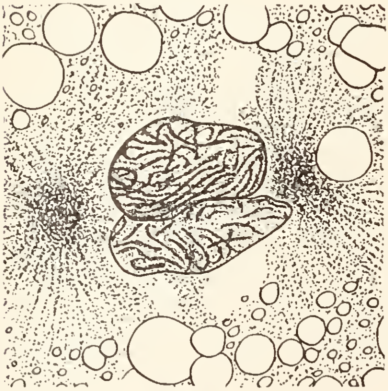

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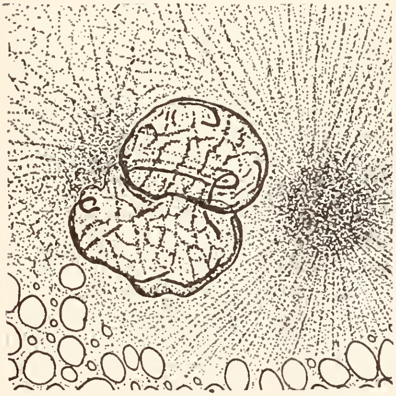

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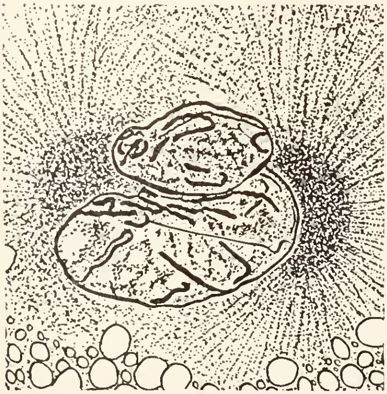

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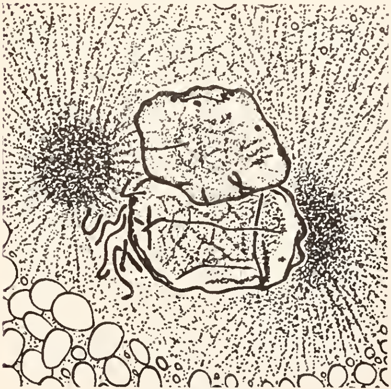

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Explanation of Plate VI.

Cryptobranchus allgeheniensis.

Fig. 27. Horizontal section through a nucleus in a late prophase of the second cleavage mitosis. A small portion of each group of chromosomes is left in each of the adjacent sections. The axis of the spindle lies in a direction nearly parallel to the plane of first cleavage. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

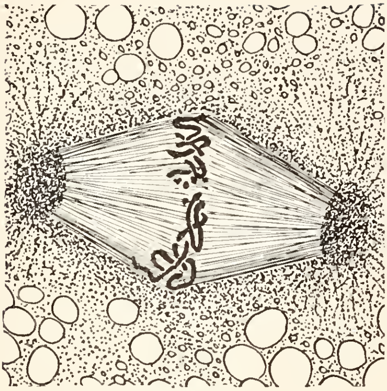
Fig. 28. Horizontal section through one of the daughter-nuclei in a late stage (early telophase) of a second cleavage mitosis. Orientation as in the preceding figure; the second cleavage furrow is not yet formed. Fixed in Zenker's fluid; stained with iron-hæmatoxylin. × 400.

Fig. 29. Horizontal section through one of the daughter-nuclei of a second cleavage mitosis; early resting stage. Part of the nucleus is left in an adjacent section where it is likewise distinctly double. The plane of apposition of the two nuclear vesicles is parallel to the first cleavage furrow, and at right angles to the second cleavage furrow which is beginning to form. Bichromate-acetic-formalin fixation; stained with iron-hæmatoxylin. × 400.

Fig. 30. Horizontal section through one of the daughter-nuclei of a second cleavage mitosis; resting stage. A portion of the nucleus is left in each of the adjacent sections where it is likewise distinctly double. The plane of apposition of the two nuclear vesicles is parallel to the plane of first cleavage, and at right angles to the beginning second cleavage furrow. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 31. Vertical section through one of the daughter-nuclei of a second cleavage mitosis; resting stage. The section is taken parallel to the plane of the second cleavage furrow which has just begun to form. Part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 32. Horizontal section through a nucleus rotating into position for the third cleavage mitosis; almost the entire nucleus is confined to this section. The second cleavage furrows are present and extend nearly to the equator of the egg. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.



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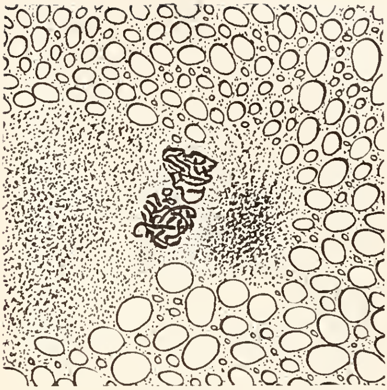

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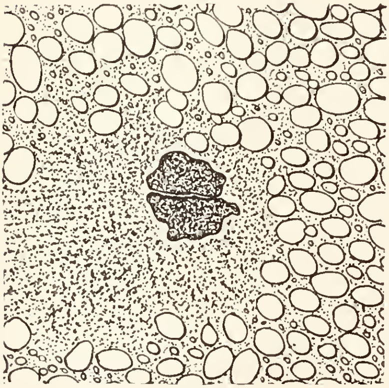

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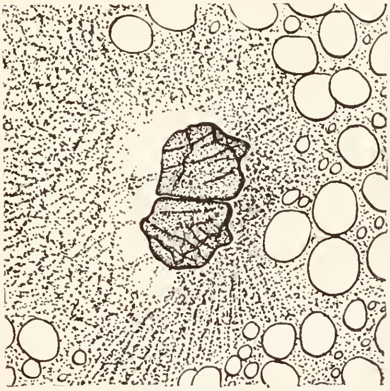

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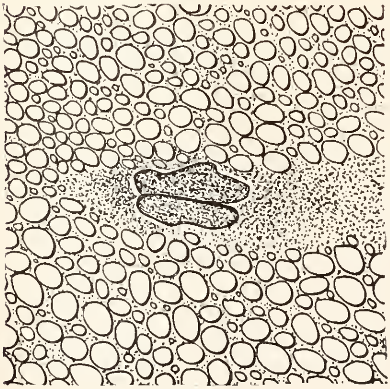

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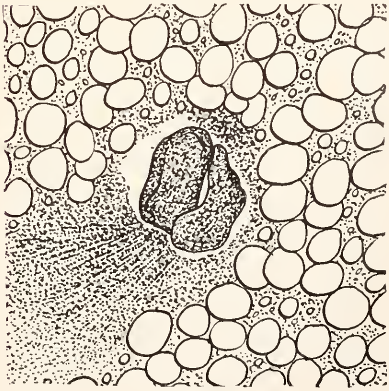

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Explanation of Plate VII.

Cryptobranchus alleghenienis.

Fig. 33. Horizontal section through a resting nucleus in position for the third cleavage mitosis. The plane of apposition of the two nuclear vesicles is nearly parallel to the *second* cleavage furrows. A considerable part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

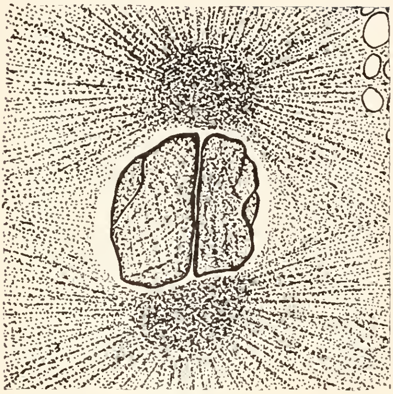
Fig. 34. Horizontal section through a nucleus in position for the third cleavage mitosis. A line connecting the two asters is nearly parallel to the second cleavage furrow. Part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 35. Vertical section through a nucleus entering upon the third cleavage mitosis. Part of the nucleus is left in each of the adjacent sections, where it is likewise distinctly double. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 36. Horizontal section through a nucleus in the late prophase of the third cleavage mitosis. A line connecting the two asters extends nearly at right angles to the first cleavage furrow. Fixed with bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 37. Horizontal section through a daughter-nucleus of a third cleavage mitosis. The two nuclear vesicles remain almost in the position in which they were formed, but two asters are present and these have taken up a position preparatory to the fourth cleavage mitosis. A line connecting the two asters extends in a direction almost parallel to the adjacent newly-formed third cleavage furrow which is vertical and intersects a second furrow almost at right angles. Fixed in bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.

Fig. 38. Horizontal section through a nucleus in position for the fourth cleavage mitosis. A line connecting the two asters extends parallel to the adjacent third cleavage furrow and almost parallel to the first furrow. Fixed in bichromate-acetic-formalin; stained with borax-carmine Lyons-blue picric-acid. × 400.



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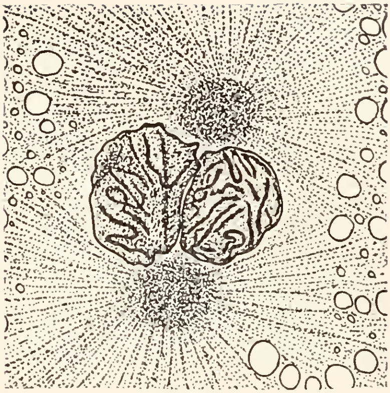

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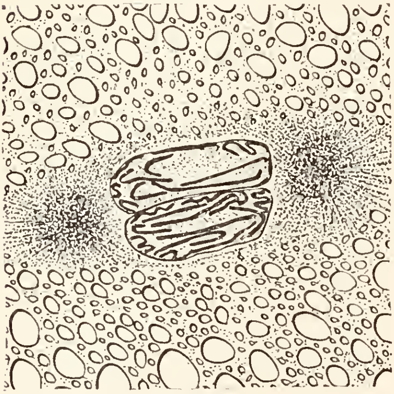

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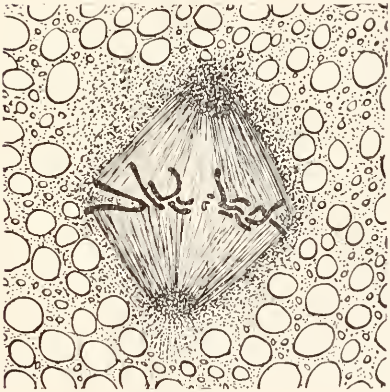

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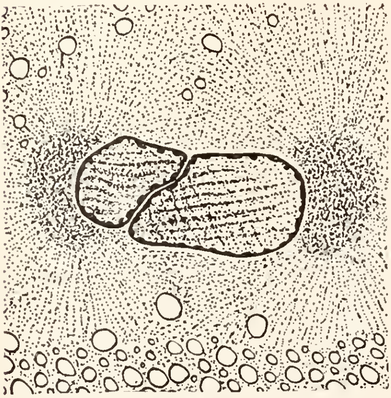

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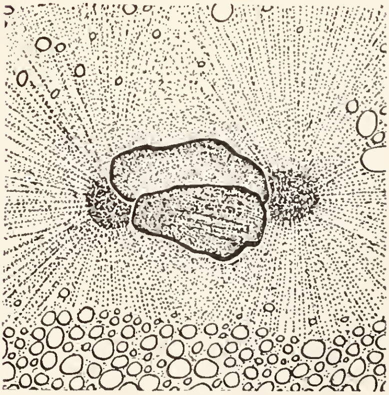

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EXPLANATION OF PLATE VIII.

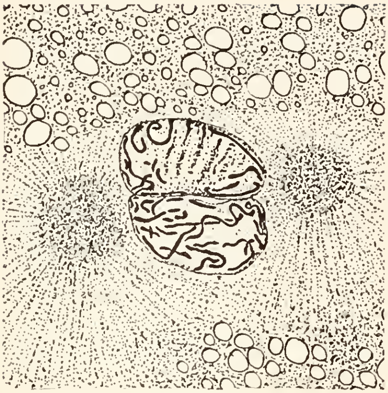
Cryptobranchus alleghenienisis.

FIGS. 39 AND 40. Two successive vertical sections through a nucleus preparing for the fourth cleavage mitosis; almost the entire nucleus lies in these two sections. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.

FIG. 41. Another nucleus found in the section used for Fig. 39; early prophase of the fourth cleavage mitosis. × 400.

FIG. 42. Vertical section through a nucleus in the metaphase of the fourth cleavage mitosis. The fourth cleavage furrow has not yet been formed. Bichromate-acetic-formalin fixation; stained with iron-hæmatoxylin. × 400.

FIGS. 43 AND 44. Two successive horizontal sections through a newly-formed daughter-nucleus of a fourth cleavage mitosis. The fourth cleavage furrow is newly formed. Bichromate-acetic-formalin fixation; stained with borax-carmine Lyons-blue picric-acid. × 400.



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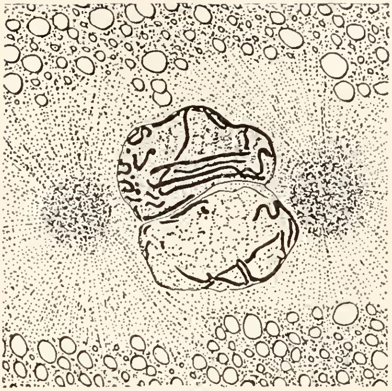

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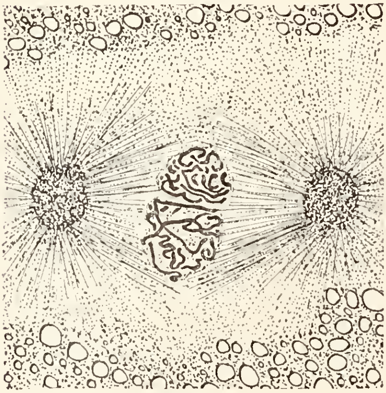

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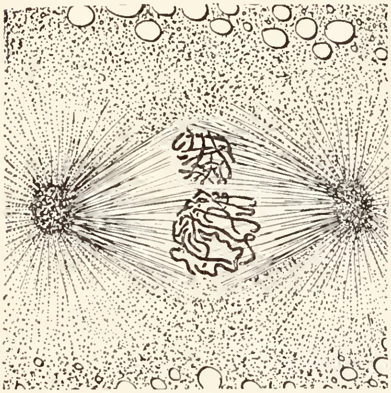

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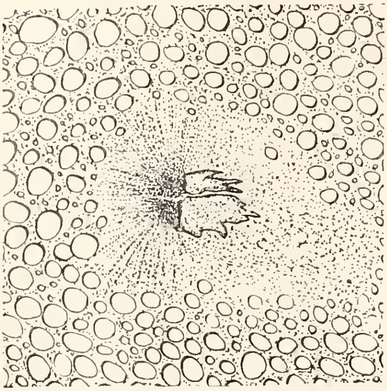

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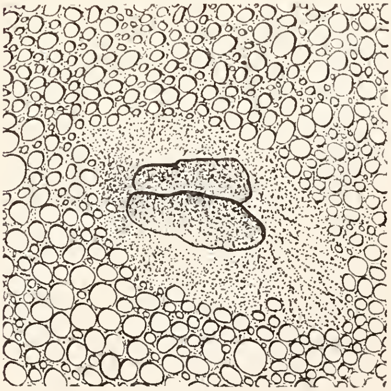

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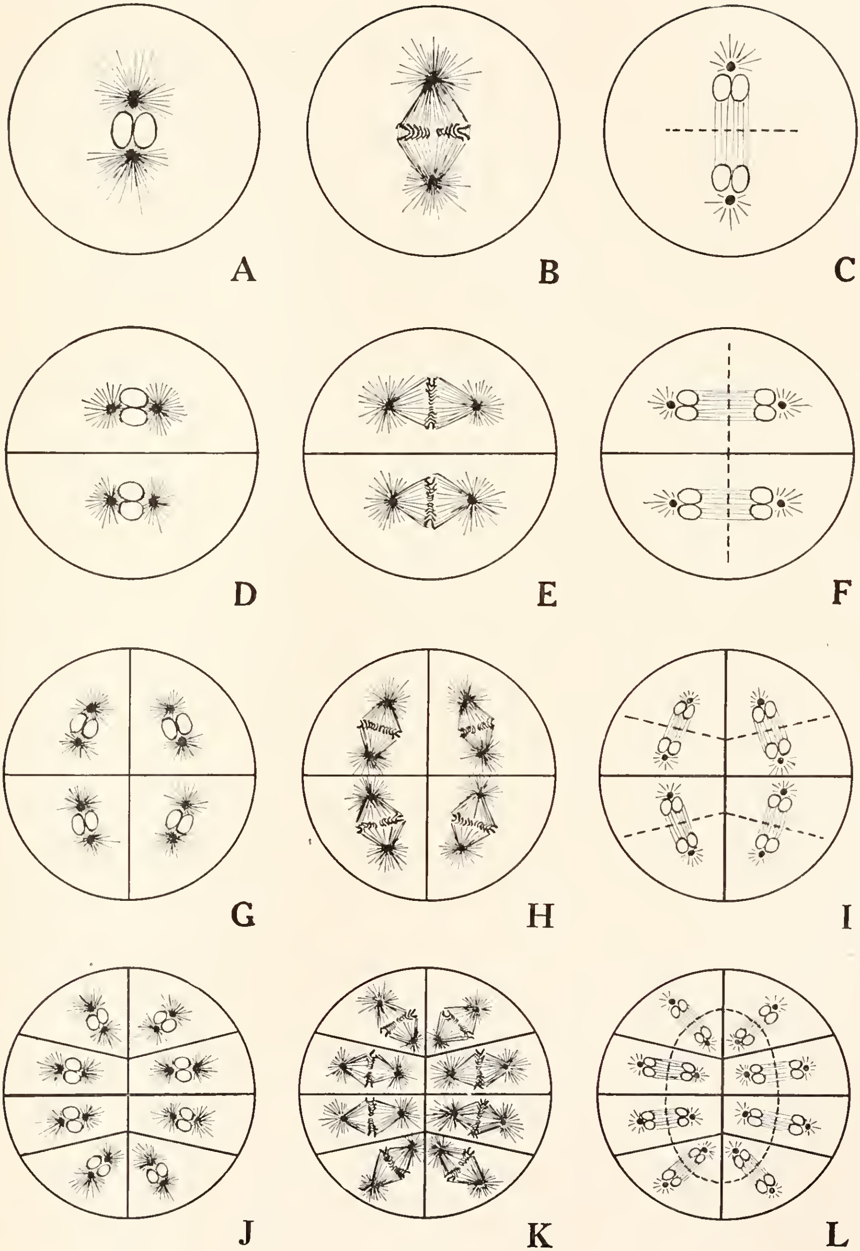
Explanation of Plate IX.

Cryptobranchus allegheniensis.

Fig. 45. Diagrams illustrating the orientation of the germ-nuclei during the first four cleavage divisions. All the diagrams represent horizontal sections, and the germ-nuclei are assumed to lie always in the same horizontal plane. The size of the nucleus is exaggerated in proportion to the size of the egg. Dotted lines represent beginning cleavage furrows; solid straight lines represent cleavage furrows which have cut to the level of the nuclei. The number of chromosomes formed from each germ-nucleus is assumed to be six, and this is probably the correct number.

A. Germ-nuclei and asters in position for the first cleavage mitosis.
B. First cleavage mitosis.
C. Newly-formed daughter-nuclei of the first cleavage mitosis.
D. Nuclear vesicles and asters in position for the second cleavage mitoses.
E. Second cleavage mitoses.
F. Newly-formed daughter-nuclei of the second cleavage mitoses.
G. Nuclear vesicles and asters in position for the third cleavage mitoses.
H. Third cleavage mitoses.
I. Newly-formed daughter-nuclei of the third cleavage mitoses.
J. Nuclear vesicles and asters in position for the fourth cleavage mitoses.
K. Fourth cleavage mitoses.
L. Newly-formed daughter-nuclei of the fourth cleavage mitoses.


A
B
C
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BIOLOGICAL BULLETIN

THE NATURE OF THE FERTILIZATION MEMBRANE OF *ASTERIAS* AND *ARBACIA* EGGS.[1]

WALTER E. GARREY.

It is generally believed that the fertilization membrane of an echinoderm egg is a distinct structure formed by the egg, and separated from it by the liquid filled perivitelline space. Views quite at variance with this current one have been advanced in recent communications; these we believe are not justified by the experimental evidence which is to be considered below.

I. Elder,[2] Kite[3] and McClendon[4] have separately maintained that the egg jelly is essential to the formation of the fertilization membrane and in one way or another enters into its composition. Harvey[5] has made this question one of special study and presents convincing experimental evidence to show that the egg jelly is not essential. He was able by repeated shaking and continued washing with sea water, to remove this jelly so completely that not a trace could be detected when the eggs were examined in India ink suspensions. Fertilizations of such eggs caused the formation of fertilization membranes, which however may be more tenuous than normally, probably due to the fact that the jelly facilitates the membrane formation by acting as a mechanical block to the diffusion of the membrane forming colloid ("membranogen") which was thus retained in more concentrated solution and consequently formed a denser membrane.

J. Loeb[6] found that hydrochloric acid would dissolve the jelly from the eggs of *Strongylocentrotus purpuratus* and that after subsequent treatment with sodium hydroxide and calcium chloride, starfish sperm caused the formation of fertilization membranes and induced development. F. R. Lillie[1] found that *Arbacia* "eggs are fertilizable" after complete removal of the jelly by Loeb's method. Loeb and Kupelwieser[2] caused the formation of fertilization membranes by treating eggs with butyric acid; these were freed from every vestige of membrane and jelly was removed by shaking, yet upon insemination they were able to form new fertilization membranes. Moore[3] noted that egg fragments, produced by shaking, formed membranes upon entrance of the spermatozoön. These observations indicate that the egg jelly is not essential for the formation of the fertilization membrane.

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[1] From the Physiological Laboratory of Tulane University, New Orleans, and the Marine Biological Laboratory, Woods Hole.

[2] Elder, *Arch. f. Entwick.*, 1913, XXXV., 195.

[3] Kite, G. L., *Science*, 1912, N.S., XXXVI., 562.

[4] McClendon, J. F., *Internat. Zeitschr. f. physik. chem. Biol.*, 1914, I., 163.

[5] Harvey, E. N., BIOL. BULL., 1914, XXVII., 237.

[6] Loeb, J., *Science*, 1914, N.S., XL., 318.

II. The facts considered in the preceding section make us doubt the validity of the hypothesis advanced by McClendon that the fertilization membrane is formed by the process of precipitation due to the contact of two colloids carrying opposite electric charges; viz., the egg jelly which McClendon states is electro-negative and another colloid (membranogen) derived from the egg which he found to be electropositive. This view is rendered quite untenable when we consider the fact that fertilization membranes are formed about echinoderm eggs by Loeb's well-known methods of inducing artificial parthenogenesis by treatment with weak fatty acids. These acids act only after penetrating the egg-jelly in the course of which event they impart to that colloid the positive electric charge of the dominant hydrogen ion; they therefore have the same charge as the colloids of the egg and the requisite conditions for precipitation of colloids do not exist.

It has furthermore been shown that in the chemical fertilization of *Asterias* eggs either acids or alkalies may be used to induce membrane formation. The electric charge of the jelly is positive when acids are used and negative with alkalis; obviously the charge is not of opposite sign to that of the egg substance (membranogen) in both cases.

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[1] Lillie, F. R., Biol. Bull., 1915, XXVIII., 24.

[2] Kupfelweiser, H., *Arch. f. Entw.*, 1909, XXVII., 434.

[3] Moore, A. R., Univ. California Pub., 1912, IV., 89.

Upon treatment with acids the egg jelly swells perceptibly and also removes the acid from solution either by adsorbing it or by chemically uniting with it. In either event the jelly becomes electropositive. The fixation of the acid is shown in the following experiments.

Ripe eggs from *Asterias* ovaries are shed in sea water and allowed to settle to the bottom of a graduated cylinder. The supernatant liquid is decanted and enough fresh sea water added to the mass of eggs to equal three times their volume. The cylinder is actively shaken and the eggs again are allowed to settle. The clear supernatant fluid containing a considerable amount of the jelly is now decanted off and an equal volume of $N/500$ butyric acid added. Fresh *Asterias* egg do not form fertilization membranes in this acid-jelly mixture but do so in a control solution of $N/1,000$ butyric acid in sea water. On the other hand fertilization membranes are formed at once in the acid-jelly mixture after the further addition of an equal volume of $N/500$ butyric acid sea water, thus indicating clearly that the initial effect of the jelly is to remove the acid from solution and that membrane formation is induced only when acid is present in excess of the amount necessary to saturate the jelly. Neglect in the observance of this precaution doubtless has been the cause of many failures to obtain good artificial fertilization by this method in case large masses of eggs have been added to small amounts of acid sea water. Loeb[1] cautioned against such a loose procedure. Under such conditions the relatively large amount of adherent jelly fixes the acid and prevents it from acting on the eggs.

III. The formation of fertilization membranes after the complete removal of the jelly indicates the origin of this structure from the egg. The following experiment furnishes a simple but equally conclusive mechanical demonstration of this fact. Fertilization of the eggs of *Asterias* either by sperm or by artificial means such as acid or heat (30–33° C.) is possible not only after maturation but also earlier, when the nuclear membrane about the germinal vesicle just begins to fade (De Lage[2]); in fact I have often observed that fertilization is possible even before the nuclear membrane begins to fade. After both polar bodies were formed I found that fertilization by any means caused the formation of the membrane between them and the egg surface, from which they were lifted and pushed away as the membrane moved outward. They are seen to lie in the saucer-like depression in the outer surface of the membrane, which results from the resistance offered by the egg-jelly to the pressure developed in the perivitelline space. After staining with dilute methylene blue, examination removes all doubt that the polar bodies are entirely outside the membrane.

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[1] Loeb, J., "Artificial Parthenogenesis" (Chicago), 1913, 69.

[2] DeLage, Y., *Arch. de zoöl. expér. et gen.*, ser. 3, IX., 285.

By so timing the fertilization that only the first polar body has been formed, it will be pushed away from the egg outside the fertilization membrane while the second polar body, being formed much later, remains at the surface of the egg, and therefore inside the perivitelline space. Similarly by fertilizing before maturation the fertilization membrane will have been formed and will have left the surface of the egg before the polar bodies are extruded. These remain at the surface of the egg and therefore within the perivitelline space. By manipulation they may be shaken free from the surface of the egg and moved about freely.

IV. Kite[1] has stated that the "so-called fertilization membrane of the egg of *Arbacia* consists of three parts, viz., the inner layer of the egg jelly which has undergone a change in refraction index, the swollen vitelline membrane, and the thin highly refractive surface layer of the cytoplasm. This hyaline layer is still very adherent to the vitelline membrane." Kite thus conceives the vitelline membrane to remain attached to the egg, to become swollen and edematous and to fill completely the entire space between the egg and the jelly which in turn is altered in refractive power to appear like a separate membrane. That the egg jelly is non-essential has been shown. The other features of Kite's conception can likewise be demonstrated to be erroneous both for *Arbacia* and *Asterias*. In these forms what appears to be the fertilization membrane is far more than the outer refractive part of an invisible thick layer of "gel" formed by a swelling of the vitelline membrane. It is a true, thin membrane formed by the egg and separated from its surface by a liquid filled space as is indicated by the following considerations:

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[1] Kite, G. L., *Science*, 1912, N.S., XXXVI., 562.

1. This new structure has entirely different permeability from that of the surfaces of either the fertilized or unfertilized eggs which are very slightly permeable to neutral salts, so that the eggs are plasmolyzed and crenated by hypertonic saline solutions. Salts however must penetrate the fertilization membrane freely to effect this change in the egg and that they do so is further shown by the fact that the fertilization membrane retains its round contour and distended condition when placed in the hypertonic or hypo-tonic salt solutions. The differences in permeability thus far demonstrated speak against the origin of the membrane from a pre-existing structure on the surface of the egg.

2. Kite's description is proven incorrect by the demonstration of the fact that the fertilization membrane is separated from the surface of the egg by a space filled with liquid and not occupied by a swollen gelatinous vitelline membrane continuous with the surface of the egg on the inside and with the egg jelly on the outside. Dr. Robt. Chambers by removing this liquid from this space for me by the use of his micro-pipette has produced a collapse of the membrane. Allusion has already been made to the fact that polar bodies extruded after the formation of the fertilization membrane may be separated from the egg surface by manipulation and are then free to assume various positions in the space; this could be possible only in liquid but certainly not if the space was occupied by any sort of a "gel."

Professor J. Loeb has shown that the fertilization membrane of echinoderms is impermeable to such colloids as egg white, shark's serum, and even tannic acid. These materials, if added to sea water, cause the thin membrane to crumple onto the surface of the enclosed egg by the osmotic extraction of water from the intervening space; but the membrane regains its original contour when replaced in sea water. This latter fact suggested to Loeb the probability that the space contains some colloid in solution secreted by the egg at the time the fertilization membrane is formed. To this colloid the membrane is impermeable; it therefore exerts its osmotic pressure in excess of that due to the salts of the sea water, serves to raise the membrane from the surface of the egg and to keep it distended.

Experiments by the writer have shown that when fertilized eggs of *Asterias* or *Arbacia* are placed in a two per cent. solution of Witte's peptone in sea water the fertilization membrane crumples instantly as in Loeb's experiments, but that when allowed to remain in the solution the membranes are again distended, indicating that they are slowly permeated by the albumoses in this preparation. Such results can only be obtained where a semi-permeable membrane, one permeable to salts but impermeable to colloids, encloses a space containing a colloidal solution.

4. Still other facts show that the space between the fertilization membrane and the egg surface is filled with a fluid and not with a gel. If *Asterias* eggs are heated to 33° C. they form fertilization membranes in from three to five minutes. By further warming for fifteen minutes they show slow amœboid movements after they have been returned to sea water at 20° C. They may migrate about in the space enclosed by the thin fertilization membrane and up close to that structure at any point. They may throw out long streamer like pseudopodia which meet with no check to their progress anywhere in the space till they reach the thin confining fertilization membrane. This over-heating process may lead to droplet formation and ultimately to partial or complete disintegration of the egg; the débris thus formed is scattered throughout the space and is never held away from the thin fertilization membrane by any gelatinous structure. Similarly if the fertilized eggs of *Asterias* are subjected to the action of diluted sea water they may be caused to swell until each presses tightly against the confining fertilization membrane and completely fills the space. Cytolysis will result in sea water which has been sufficiently diluted and in this case, as in that of cytolysis caused by prolonged warming, the granular material completely fills the perivitelline space which may be more than twice the original diameter of the egg. When extra-ovates rupture through the fertilization membrane after treatment with hypotonic solutions, the egg is drawn over tightly against the inner surface of the membrane at the point of rupture, the thinness of the membrane being evident at the constricted neck between the extra-ovate and the main egg mass.

Again, in the formation of the blastula the cells range up close to the inner surface of the fertilization membrane and completely obliterate the interior space giving a further indication that neither a thickened vitelline membrane nor any "gel" is there.

These demonstrations may be made immediately after fertilization so that there is no necessity to assume that the vitelline membrane or a gel in the perivitelline space has had time to undergo liquefaction.

The above facts and others which might be referred to all indicate the fluid character of the contents of the space between the fertilization membrane and the egg surface and serve to substantiate the conception of the fertilization membrane stated in the opening paragraph of this communication. In the case of both *Arbacia* and *Asterias* the fertilization membrane is a structure which arises at the surface of the egg and is subsequently lifted from it and distended by fluid. Depending upon the time of fertilization of *Asterias* eggs relative to the formation of the polar bodies these structures may be outside the fertilization membrane, inside the perivitelline space or the first outside and the second within the fertilization membrane.

Heilbrunn[1] has reviewed the subject of membrane production by *Arbacia* eggs under the influence of various chemicals. He concludes with Herbst and others that in this form the membrane exists preformed on the surface of the unfertilized egg and that fertilizing agents merely cause its elevation. He gives evidence in support of the view that this elevation is the result of lowering of surface tension and swelling of proteins beneath the membrane. In the light of our findings it is clear that the fluid character of the contents of the intra-membranal space and the progressive increase in its bulk as the membrane is lifted away from the egg indicate that the colloids are in solution and that the process involves the osmotic attraction of water.

It is not yet demonstrated that a membrane exists preformed on the surface of unfertilized *Asterias* egg and the formation of a membrane on a fragment of *Arbacia* egg as described by Moore (*loc. cit.*) are facts which speak, in these instances, for the formation of the fertilization membrane *de novo*.

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[1] Heilbrunn, Lewis V., BIOL. BULL., 1913, XXIV, p. 343.

THE EFFECT OF ULTRA-VIOLET LIGHT RAYS UPON THE DEVELOPMENT OF THE FROG'S EGG.

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II The Artificial Production of Folded (U-shaped) Embryos.

This paper is the second of a series dealing with an investigation of the nature and mechanism of causation of certain abnormal, developmental results obtained by raying certain restricted areas of the fertilized ovum of the frog by means of ultra-violet light rays. Since the first paper dealt with the production of a single type-defect, spina bifida, the possibility of production of another constant and fixed experimental result, a folded or U-shaped embryo, justifies the separate consideration of that result in this contribution. Once again the value of the physical method of attack upon the complex problem of developmental reaction is demonstrated. In this problem we are concerned with a readily controllable and constant, causative agent on the one hand and a uniform embryological defect on the other. This one technical condition permits of a more thorough analytical inquiry into the mechanism of production of this developmental defect. In addition to this consideration, however, as is to be expected the experiment gives further insight into the genetic constitution of the fertilized, undivided ovum, and, more specifically, helps in the identification and location of the organ forming substances and anlagen. The apparatus used for the experiment was that detailed in the preceding paper. Some of the work was done, however, with an apparatus for the use of which I am indebted to Mr. W. S. Andrews, of the General Electric Company at Schenectady. With both forms of apparatus, the surface area concentration of the rays was increased through the use of convex quartz lenses.

The eggs used were those of the common species of frogs found around Ithaca and also Schenectady, N. Y. These were collected early in the morning, as soon as possible after laying. They were brought into the laboratory, divested of their jelly, and rayed while still in the undivided or two-celled stage. A thin tin-foil diaphragm perforated by a hole three tenths of a millimeter in diameter shielded most of the surface area of the eggs from the rays but permitted a relatively small portion of that area to be influenced. After raying, the eggs were transferred to a jar containing 1,000 c.c. of tap water in which they were permitted to develop, the water being changed frequently.

Physicists have definitely established the fact that ultra-violet light rays possess a very slight penetration capacity. We are justified, hence, in assuming that the chemical alterations produced by the rays acting under these conditions were restricted to a superficial layer of protoplasm of the egg in the restricted area mentioned. The interpretations presented in this paper are made, accordingly, upon that basis.

The surface area of the egg subjected to examination in this experiment is best demonstrated by a reference to Fig. 1. A two-cell ovum is shown with the animal pole uppermost. The broken line marks out the area investigated. As is to be seen this extends from the region of the equator up towards the animal pole but is exclusive both of the pole itself and of a narrow median strip which connects the latter with the equator. As can be seen this region is exclusive of the gray crescent. Different portions of this area were rayed successively with the three tenths millimeter beam of light. As a result, when development

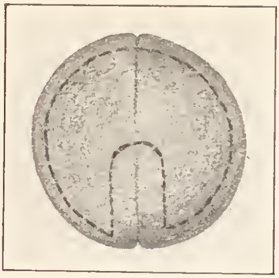

FIG. 1.

was permitted to progress without further interference, the long axis of the trunk of the embryos was invariably bent towards one or the other side. The level of this bending or flexure of the trunk depended directly upon the location of the area influenced by the light.

Since the jelly which envelops the eggs had been found in the spina bifida experiments to be impervious to ultra-violet light rays, care was taken to remove it prior to the experiments· As a result a marked increase in the percentage of positive results followed the raying. Where, as was the case more particularly with green frog's eggs, the jelly could be removed almost completely, about every egg rayed developed an abnormality which conformed to this general, U-shaped type. Three type-experiments may be presented for the purpose of demonstrating the constancy of results. On May 27, 1914, 9 eggs in the two-celled stage were rayed, each for 1 minute. One died before development had progressed more than 24 hours. Of the 8 remaining all were U-shaped. Three of these were permitted to develop until the tail and head were well formed. The others were killed at earlier developmental stages. On June 18, 1914, 25 eggs were rayed, each for 30 seconds. These were in the one- and two-celled stage. Various portions of the prescribed area above the equator were influenced. Three died within 24 hours, one lived until the neural groove had disappeared and presented a marked curvature of the trunk. All of the remaining 21 were U-shaped. On June 23, 1914, an exposure of 19 eggs in the one- and two-celled stage was made, each for 30 seconds. Eight of these were rayed in the designated area, and 11 along the equator. One of the eight died early, of the remaining 7 all were U-shaped. The 11 others demonstrated spina bifida.

The inference seems justifiable, therefore, that the constant type of defect produced is the result of a constant and uniform degree of alteration of the superficially-placed egg substance in the area illuminated. Just what the nature of this effect is we are at present unable to state. It would appear that the protoplasm, using this term in its broadest sense, had been modified in some chemical way to a degree which not only rendered it unfit for participation in the subsequent chemical ontogenetic processes of which it normally was a part but, in addition, caused its presence to act as a mechanical hindrance to the developmental shifting of anlagen.

Studies of the action of ultra-violet light rays have produced proof that proteins, carbohydrates, and lipins may be chemically altered by means of this form of energy. To review the experimental results briefly: Massol and Kluyver have ascertained that starches may be altered, and Stoklasa, Zdobnický, Chauchard and Mazoné, and Pougnet that they may be ultimately broken up. Diastase may be liberated in plants (Maquenne and Demoussy). Bierry, Henri, and Ranc have inverted cane sugar. Agulhon, Maurain and Warcollier, and Raybaud presented evidence that the action of enzymes in the presence of oxygen was weakened and destroyed by ultra-violet light. Furthermore, albumen may be coagulated (Bovie) and the iodine content of fats may be lowered (Roemer and Sames). Thus we may reasonably infer that ultra-violet light rays are capable of bringing about certain chemical modifications of the protoplasm of the cell to all or to part of which we may attribute the abnormality in development noted. The evidence is lacking at present, however, which might enable us to associate this developmental result with a specific alteration of protein, of carbohydrate, or of lipin.

It was pointed out in the preceding paper that the superficial mass of altered protoplasm situated between the approximating neural ridges prevented their normal fusion with the consequent formation of a complete neural tube. The condition of spina bifida resulted, therefore, as a direct result of the mere mechanical interruption of this process. The action of the altered protoplasm was owing entirely to its passive function as a mass which mechanically interfered with the medianward, migratory movement of the hemineural anlagen. At least, in this one factor could be found sufficient evidence as the causative condition of this abnormality.

In the present instance of the U-shaped embryo, however, the inert mass was located lateral to and above the level of the hemineural anlagen. It could not, therefore, by virtue of interposition mechanically interfere with the approximation of the neural tube-halves. Because of its lateral position and close relationship to one of these halves it might exert, however, a restraining influence upon the rate of migration of the tube-half of the corresponding side of the egg. This retardation of the shifting of the hemineural anlagen on the affected side induced a correspondingly exaggerated migratory process in the anlagen upon the unaffected side of the egg. As a result of this loss of coördinated, migratory movement, the anlagen upon the affected side had not yet reached the median plane before fusion took place with the unaffected anlagen by reason of their exaggerated migration. The normal angularity of the hemineural long-axis persisted more or less completely, therefore, in the adult tadpole as a result of this process. This was expressed by the curvature of the trunk invariably towards the affected side. The location of this flexure, as was stated above, was directly dependent upon the segmental level of the affected area of protoplasm.

It is a singular fact that the histological sections failed to demonstrate a single gross or microscopic defect in these embryos. The viscera developed normal in shape and in size. No defects could be recognized in the muscle segments. The mass of these in the concavity of the flexure was estimated roughly to be the same as that of the corresponding segments on the convex side of the body though their shape was necessarily distorted to fit the concavity. The yolk mass of the trunk of the embryo was completely utilized in the differentiation of the tissues and in the metabolical processes of the embryo. There was no evidence at any time that a portion of this yolk mass had been rendered completely inert by the rays and consequently excluded from participation in the normal chemical developmental processes of the body.

The action of the rays inferentially, therefore, had not induced such chemical changes as would permanently eliminate the substances affected from participation in .the normal organo-genetic or somatogenetic processes. But the rate of chemical modification of these substances in participation in the normal metabolical processes of the embryo was, however, retarded. We may find in this retardation of chemical participation additional evidence for the causation of the check of the normal lengthening process of the embryo on the affected side. The opposite side progressed approximately at its normal, but comparatively greater, rate. As a result, the flexure of the neural tube increased proportionate to the developmental progress of the embryo. In other words, as an embryo increased in age the angle formed by the trunk with the tail became more and more acute until a condition was arrived at finally in which the long axis of the trunk lay parallel to that of the tail.

The sketches presented with this paper are intended merely to demonstrate the general morphological and histological features which are characteristic of the development of these abnormal, U-shaped embryos. It may be stated, however, that no great attempt has been made to make them faithfully representative of all of the histological details of the tadpoles. In many instances the drawings are composite. In every instance, however, the basis of each drawing has been an actual specimen. The figures are to be interpreted, therefore, as merely type representatives of the numerous examples which are illustrative of the peculiarities of this form of abnormal development.

Fig. 2 represents an embryo in the early stages of development viewed from the dorsal aspect. The deep furrow which penetrates from the left, to and beyond the center of the embryo mass represents the line of apposition of the two portions of the right flank of the embryo which is so bent upon itself that the right surface of the tail region is in contact with the right surface

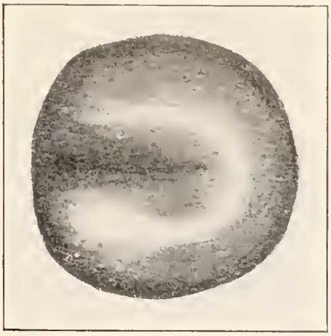

FIG. 2.

of the head region. Actual continuity of the tissues, however, does not exist. The cephalic end of the embryo, which is represented below and to the left of the sketch, is distinguished by its greater transverse diameter. The area of protoplasm in this specimen affected by the rays lay in the concave bend of the trunk at the extremity of the cleft. It will be noted that this flexure is sharply restricted to a relatively small portion of the embryo. Cephalic to and caudal to this level, both the head-end and the tail-end of the embryo are, respectively, of normal shape.

Occasionally, the two neural tube-halves fail to unite in the caudal region, bringing about a condition which is demonstrated well by Fig. 3. In this the yolk mass is represented as projecting to the right between the caudal extremities of the divided neural tube. This is an early developmental feature in the production of the condition of spina bifida, the divided condition of the neural tube-halves persisting in the adult free-swimming tadpole. The bend in this particular embryo is represented as occurring high up in the trunk region. The cephalic end of the embryo is shown with its right surface resting against the right neural tube-half caudally. As was noted in connection with the preceeding figure, so is it likewise true of this, both cephalic to and caudal to the angle of the fold, aside from the features of spina bifida, the appearance of the embryo is practically normal. The stages of development represented by these two figures are relatively early, consequently but little of the ordinary features of external configuration of the embryo can be made out.

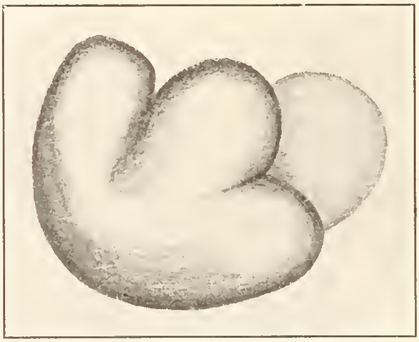

FIG. 3.

A still later stage of development demonstrating a more marked differentiation of the tail from the head region is shown by Fig. 4, while the tadpole in Fig. 5 represents the external features of these tadpoles during the free-swimming period. In this latter picture, the sucker, the eyes, the external gills and the tail fins are readily made out. The sharpness of the fold in the trunk in both instances indicates a restricted localization of the alteration produced in the protoplasm of the developing ovum.

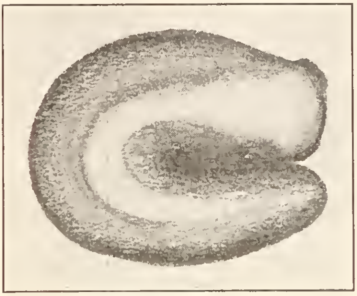

FIG. 4.

These creatures are unable to straighten out the flexure of the trunk. They swim, as was noted before, by means of ordinary tail movements, the vigor of which is not impaired in any single instance. As can readily be understood by reference to the figure, however, the abnormal position of the caudal long-axis, with regard to the long-axis of the head and remaining portion of the trunk, is such that the swimming movements of the tail tend to force the embryo about in a circular or spiral

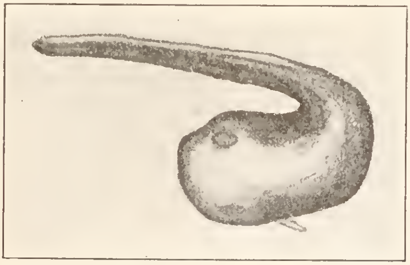

FIG. 5.

direction. Because of the condition of fixity of the bend progression directly forward is necessarily out of the question.

The deformity represented by Fig. 6 is illustrative of the second mechanism of production of the folded embryos. The concave side of the neural tube, to the right in the figure, is occupied by irregular bulgings of the epidermal surface of the embryo. When the development of embryos conforming to this type is followed from day to day, it is noted that the flexure of the neural tube becomes greater from day to day. The apparent occasion of this is referable chiefly to two factors; one, the normal but relatively more rapid growth of the normal myomeres to the left of the longitudinal axis of the body, and the other, to the relatively, and actually slower, growth of them upon the concave side of the median body plane. Histological studies demonstrated as well that differentiation in the myomeres upon the affected side was somewhat retarded.

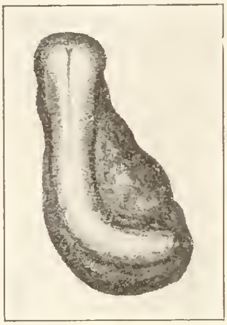

FIG. 6.

There are, therefore, these two general developmental mechanisms either of which leads to the production of a U-shaped embryo. In one the angularity of the bend is constant from the time of its first appearance in the embryo, whereas in the other this angularity increases with the succeeding stages of differentiation and growth of the anlagen. The end result is identical, however, in both instances both from the morphological and the histological standpoint. Furthermore, in both instances the segmental relationship of the bend to the area of protoplasm altered in the ovum is the same.

The broken lines shown in Fig. 4 bear numerals which correspond to the planes of section represented by the succeeding figures, 7 and 8. The cross-section represented by 7, therefore, passes through the embryo at the level of the fold in the neural tube. The two halves of the yolk mass with sections of the enteron which belong, the one to the cephalic end and the other to the caudal end of the embryo, are shown separated from each other by a deep cleft lined by ectoderm. Dorsal to the enteron lie two sections of the notochord with the myomeres sectioned in their long-axis between them. Between and dorsal to these two portions of the notochord, the oblique section of the bend in the neural tube, demonstrating the neurocele as well, is to be seen. The level represented by Fig. 8 includes the region of the brain and subjacent pharynx to the right and the trunk to the

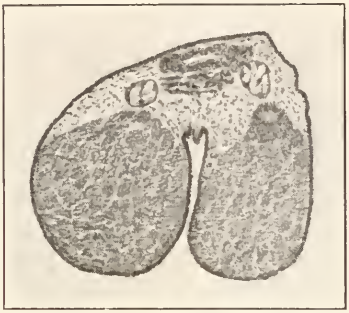

FIG. 7.

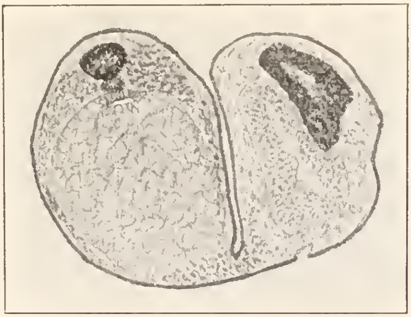

FIG. 8.

left. Both portions of the embryo are connected with each other by a narrow strip of mesenchymal tissue at the bottom of the epidermal cleft. The notochord is to be seen only in the left half of the embryo with the myoblastic tissue to either side of it, the enteron ventral and the neural tube dorsal. The stage is so early that most of the yolk cells are in an undifferentiated condition.

Figs. 9, 10 and 11 are sections of one and the same embryo. In this the bend of the trunk occurred at about the level of its middle segment. The section-knife encountered both the neural tube and the notochord at their curvature. The undifferentiated yolk-mass of the two limbs of the U are connected by a relatively slender commissure-like mass of yolk cells and of mesenchymal tissue. The section of Fig. 10 taken at about the level 8 of Fig. 4 demonstrates on the left the transsected notochord with the cephalic end of the definitive spinal cord corsal to it, both imbedded in myoblastic cells not yet well differentiated. A section of notochord and of spinal cord with the

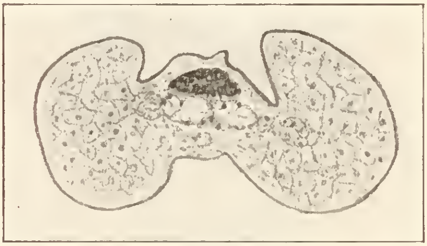

Fig. 9.

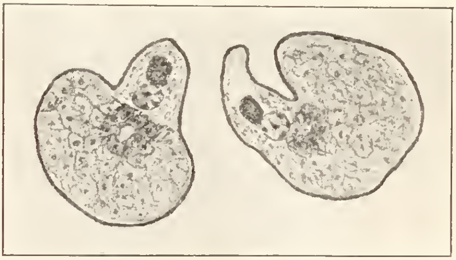

Fig. 10.

enteron ventrally placed to them are to be seen in the right half of the sketch. There is no indication at this early stage of a beginning formation of the anlagen either of the liver or of the pancreas. The section represented by 11, however, passes through the head region at the level of the eyes. To the left in this sketch there can be identified the cephalic portion of the neural tube with its well-differentiated walls showing a degree of stratification. The neurocele appears clear and contains no cell detritus. The neuroblasts do not demonstrate any pathological features. A thin layer of mesenchymal tissue intervenes between the roof of the neurocele and the epidermis. Immediately ventral to this section of the brain cavity the knife has encountered another portion of the normally folded brain stem with its ventricle and, projecting to either side from its ventral aspect, a portion of the optic commissure and stalks proceeding laterally to the optic cups. The cytological appearance of these structures presents no departures from normal. The cavities are free from cell detritus. The lens of the eye on both sides is in its normal position. Stratification of the walls of the optic cup, of the stalk and of the brain vesicle have progressed to their normal relative degree. Immediately ventral to these structures in the median line is shown a section through the pharynx, the cells of which, as well, show none but normal features. The right half of the sketch demonstrates the prominent caudal fin, transected neural tube and notochord, and the caudal extremity of the enteron, the last embedded in the dorsal region of the yolk

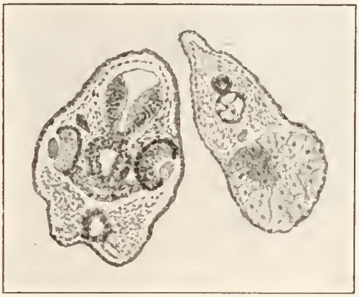

FIG. 11.

mass. The mesenchymal tissue to either side of the neural tube and notochord presents normal features.

Comparable to the levels represented by Figs. 7 and 9 is the section represented by Fig. 12. Here again the flexure of the neural tube lies dorsal to the notochord sections. Immediately ventral to the latter the forms of the elongated myoblasts are to be made out. A relatively early stage of development of the enteron is evidenced by the condition of the yolk cells, the symmetrical masses of which are separated by the deep epidermal cleft.

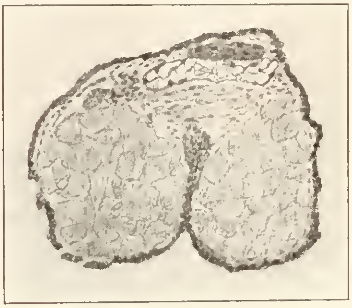

Fig. 12.

In the succeeding four figures, 13, 14, 15 and 16, embryos are represented in each of which the angularity of the flexure was not sufficient to bring the long axis of the two body portions parallel to each other. In each instance, the cephalic extremity

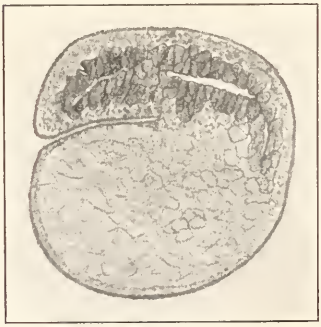

Fig. 13.

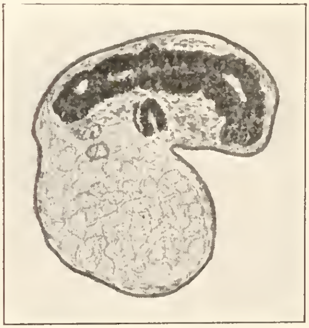

Fig. 14.

of the embryo projected from the trunk at approximately a right angle. Consequently the neural tube is sectioned in the head region parallel to its long axis, but in the trunk region at right angles to its long axis. In Fig. 14, for instance, the head end of the embryo projects to the right. The walls of the brain vesicle demonstrate no abnormal features. Because of a certain amount of twisting of this extremity but little of the pharynx appears in the section upon the ventral aspect of the brain stem. At the left extremity of the neural tube an almost perfect transection of the cephalic end of the cord is shown. The notochord with the transected enteron and the yolk-cell mass are represented immediately ventral to the tube. In Fig. 15, the cephalic

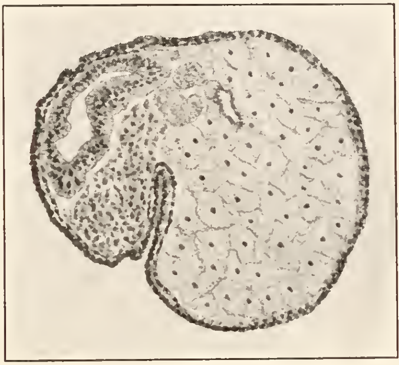

FIG. 15.

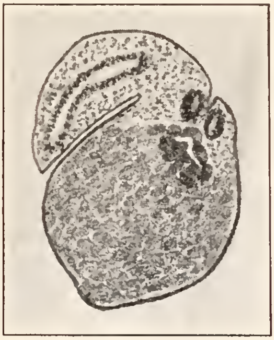

FIG. 16.

extremity of the embryo is shown above to the left resting upon the trunk of the embryo. Here again approximately the long axis of the brain tube has been encountered by the section knife. Stratification of brain walls has begun. The enteron together with the notochord lie to the right in the sketch. The mesodermal cells constituting the wall of the pharynx lie immediately ventral to the brain tube. Fig. 16 presents these same general features and, in addition, a bifid appearance of the caudal portion of the neural tube upon the right side of the sketch. The ectoderm folds in between the two tube-halves each of which, while presenting an entire tube, demonstrates its derivation by reason of the thinning of its medial wall. This appearance has been previously referred to in the paper on the condition of spina bifida.

It is to be noted that not one of the histological sections, which are presented, as was mentioned before, as merely typical instances of the appearance of the tissues during the different developmental stages of the embryos, presents any indications whatever either of cell or of tissue disorganization. There is no indication of the presence of degenerated areas of protoplasm either in the yolk mass or in the normal body cavities, nor can there be found any degenerated or extruded nuclei. The organs of the embryo such as the enteron, the brain, the cord, the myomeres, the notochord, and so on, seems to be normally formed and are of the normal size. So far as can be ascertained through the study of longitudinal sections of the myomeres at the level of the bend in the trunk the cause of this phenomenon is not, apparently, referable either to a degeneration or to a falling out of myomeres upon the concave side of the trunk. As might be reasonably expected, however, the myomeres upon this side have a form which departs from the normal in so far as the segments conform to the concavity. The corresponding segments on the opposite side of the fold are consequently relatively elongated. The normal morphological appearances of the tissues argued for a normal physiological activity as well, and this seemed to be attested by the movements and general vitality of the tadpoles. None of these tadpoles was permitted to live, however, to the period of transformation.

In the preceding paper on "The Artificial Production of Spina Bifida" it was noted that, apparently, the proanlagen and anlagen of the embryo were restricted to the pigmented hemisphere of the egg, that the raying of a small localized surface-area of the yolk hemisphere or of the region of the quarter produced invariably the condition of spina bifida in the embryo. It was noted in these embryos, however, that indications of the destruction of cell groups or of definite areas of protoplasm in the adult tadpoles were absent. No gross structural defects of organs aside from the bifid character of the spinal cord were demonstrable. Where the dosage of ultra-violet light ray energy was sufficiently great, however, a mass of protoplasm equal in size to the superficial area affected was extruded from the body of the embryo. This was observed as well in this present experiment. Under such conditions naturally the developed embryo demonstrated both an organic and a structural defect. When the dosage is reduced in amount, however, it is demonstrated both by these and by the spina bifida experiments that the possibility exists for the production of a developmental defect the structural cause of which may not be present or recognizable in the adult. The absence of atypical cytological characters as well as organic defects and of exovates must force us to the conclusion that either such did exist for a longer or shorter period during the development of the embryo and subsequently became incorporated into the structure of the body as the result of an elaborate but delayed chemical transformation, or that there ensued upon the raying a transitory suspension of the physiological activity of the cells affected with a later complete resumption of that activity. Either conclusion must force us to presume, however, the presence of a chemical alteration either of cell content or of cell mass as a direct result of the raying. Indeed, it would be rather difficult to contend that no change was brought about in the chemical composition of the protoplasm in the area affected by the rays.

Since it has been definitely proved that protein substances, carbohydrates and lipins may be altered to some degree in their chemical constitution by this form of energy, it does not appear to be unreasonable to infer that one or all of the corresponding groups of substances may be susceptible to the influence of the rays while still in the living ovum. Exovation as the direct result of the application of a great amount of energy may be cited as an instance of this alteration. The normal histological features of the embryo, and of the adult tadpoles, however, argue the presence of a process of restoration or of regeneration during the time of development of the embryo of the changes which were brought about through the activity of the rays. It might be assumed, accordingly, that the chemical modification thus artificially produced is gradually rectified during the developmental cycle but during the early stages of this process the substances involved are incapable of entering into their normal functions in the chemical elaboration of the pro-anlagen and anlagen. The resultant action of these altered substances might be interpreted, therefore, as that of an inert body which serves, as was seen to be the case with the spina bifida embryos, as a check to the gross, mechanical shiftings characteristic of the normal genesis of the anlagen. The absence of an area of altered protoplasm or of organic defects in the adult tadpole is the basis for the logical argument that the chemical readjustment of the protoplasm, altered by the rays, is ultimately completed to the degree that this protoplasmic mass does enter finally into its usual function in the elaboration of the normal anlagen.

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DIFFERENTIAL SUSCEPTIBILITY AS A BASIS FOR MODIFICATION AND CONTROL OF EARLY DEVELOPMENT IN THE FROG.

A. W. BELLAMY.

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I. INTRODUCTION AND GENERAL STATEMENT OF THE PROBLEM.

Observations on vertebrate teratology date almost from the beginning of descriptive biology, though active accumulation of experimental data on the frog especially, began little more than a quarter of a century ago. Among the early papers on the subject may be mentioned those of O. Hertwig (1892, 1894 1895, 1896); Gurwitsch (1895, 1896); Morgan and Tsuda (1894). These and other workers have studied the early development of certain teleosts, amphibia and birds under the influence of such physical factors as: gravity, mechanical pressure, temperature, light, electricity and magnetism, X-rays, radium emanations, atmospheric pressure, and a wide variety of chemical substances.

It is perhaps sufficient, here, merely to mention the essential similarity of terata produced by widely different methods, such, *e.g.*, as high and low temperatures, many different chemical agents, and heterogeneous hybridization. Equatorial gastrulation, embryos with permanent yolk plugs, with spina bifida, microcephalic forms and many other abnormalities in the frog have been produced by nearly all the methods known to experimental teratology. For instance, microcephalic frog embryos with permanent yolk plugs, and spina bifida have been produced by exposing different stages in development to such external agents as: high temperature (Jenkinson, 1909, p. 98, fig. 49); 0.6 per cent. NaCl (Hertwig, 1894, p. 315, 316, 1895, plate XX., figs. 5, 6, 7, 10, 12, 15, 16, et al); fertilizing "over-ripe" eggs (Hertwig, 1892); strychnine (Gurwitsch, 1896); sugar solutions (Bataillon, 1901, figs. 2, 5); Jenkinson (1906) produced these same types (among many others) in a number of isotonic solutions of Na_2SO_4, K_2SO_4, $NaNO_3$, urea, sugar solutions, et al.

Cyclopia in *Fundulus* has been produced by physical and chemical agents and by hybridization. Stockard (1909) obtained this anomaly with the aid of magnesium chloride and for a time ascribed a specific cyclopia producing property to this salt. Later, Stockard produced cyclopic monsters with alcohol and other substances and was forced to abandon his theory of specificity. McClendon (1912) had no difficulty in producing cyclopia in isotonic solutions of NaCl, LiCl, $MgCl_2$, NaOH and in alcohol, and other substances. McClendon is somewhat at a loss to explain his results but sums up the facts in these words: "We need only suppose that the cells between the eye anlagen are more easily affected than other cells of the embryo, to explain the action of the solutions." Werber (1915, 1916, 1917) obtained all the known types of terata in *Fundulus* by using substances usually found among the products of destructive metabolism, viz., butyric acid, acetone, urea, lactic acid, etc. In the concentrations used he found butyric acid and acetone, especially the latter, somewhat more effective than the other substances in the production of monstrous development. Werber looks upon abnormal development as due to a "blastolytic" action of the deleterious agent upon the primordial regions of the eggs, especially those at the anterior end of the body. Kellicott (1916) has secured the usual wide variety of terata in *Fundulus* simply by subjecting the early egg stages to the action of low temperature. He is inclined to look upon teratogenesis as being due in some way to a "disorganization" of the egg materials. Newman (1917) noticed the same range of abnormalities among his heterogeneous *Fundulus* hybrids and applied Child's axial gradient theory to the interpretation of these and other terata seen in teleosts produced by chemical and physical agents.

Perhaps enough has been said by way of a preliminary statement to indicate the essential similarity of experimentally produced monsters regardless of the methods employed in obtaining them. Likewise a little reflection on the various theories advanced to account for monstrous development makes apparent the lack, hitherto, of an adequate basis for a rational interpretation of them.

It is the purpose of this report to attempt an analysis of the problem of physiological axiation and teratogeny in the frog from a physiological point of view. It considers the general problem of physiological axiation, the origin of polarity, and presents data on the modification and control of early development in the frog on the basis of differential susceptibility.

II. Material and Methods.

1. *The Susceptibility Method.*—Observations on many species of animals and plants have shown that definite and characteristic differences in susceptibility to a wide variety of physical and chemical agents, within certain limits of concentrations or in-

tensities of action, exist in relation to the physiological axes.[1] These differences in susceptibility may be demonstrated in several ways: first, as differences in survival time of one region of the egg or embryo as compared to other regions, under conditions severe enough to kill without permitting acclimation to occur; second, as differences in the degree of inhibition of growth and development, or in certain cases as differences in the degree of acceleration of these processes; third, as differences in the rate or degree of acclimation to a certain range of less severe conditions; fourth, as differences in the rate or degree of recovery after temporary exposure to conditions that inhibit development.

These differences in susceptibility determined in these different ways are all expressions of the fact that a "differential susceptibility" to the action of external agents is a characteristic feature of physiological axes in both plants and animals so far as they have been examined with reference to this point. This "differential susceptibility" appears according to experimental conditions as differential disintegration associated with death, and as differential inhibition, acclimation or recovery in development.

The high degree of uniformity in the susceptibility relations in different organisms, both animals and plants, to a wide variety of agents and conditions, viz., cyanides, anesthetics, acids, alkalies, various salts, certain alkaloids, physical conditions such as extremes of temperature and certain conditions that may be termed negative, such as lack of oxygen, indicate that in their general features these susceptibility relations are independent of specific qualitative differences in the protoplasm of different forms. If this is the case they must depend upon quantitative differences of some sort which are common at least to all forms in which these differences in susceptibility have been shown to exist.

So far as present knowledge goes, the facts indicate that susceptibility, in the sense in which the term is used here, is associated in some way, directly or indirectly, with the rate of certain fundamental metabolic reactions, such, *e.g.*, as oxidation-reduction processes, and the protoplasmic conditions associated with them. Considerable evidence has been accumulated which indicates that in general the susceptibility to lethal or strongly toxic conditions and the capacity for acclimation to less severe conditions and for recovery after temporary exposure —all vary more or less directly with, though not necessarily proportionally to, the rate of fundamental metabolic reactions. This does not mean that all the agents employed act directly upon these reactions in every case or that all act in the same way upon protoplasm. It probably means merely that living protoplasm is a system of more or less closely associated and interdependent reactions and conditions so that no essential factor in this system can be altered beyond a certain degree without involving the system as a whole.

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[1] The extensive literature on this subject, previous to 1915, mostly the work of Child, is treated in two of his recent books (1915*a*, 1915*b*) to which the reader is referred for specific references. Other references are: Behre (1918); Child (1916*a*, 1916*b*, 1916*d*; 1917*a*, 1917*b*, 1917*c*, 1917*d*; 1919*a*, 1919*b*); Child and Hyman (1919); Hyman (1916*a*, 1916*b*; 1917*a*, 1917*b*; 1919).

But, however the facts of differential susceptibility may finally be interpreted, concerning the facts themselves there can be no doubt. They have afforded a means not only of demonstrating characteristic differences in physiological conditions along the axes of organisms but also of modifying and controlling development in definite ways through these differences. This study of the development of the frog was undertaken in the attempt to determine whether, and to what extent, the susceptibility method could be used in modifying and controlling development in a vertebrate, as they have been used in investigations of the physiology of development in certain invertebrates.

I am under obligation to Professor C. M. Child, in whose laboratory this work was done, for essential aid in the way of suggestion and criticism, and to Dr. L. H. Hyman for many thoughtful suggestions. Work on amphibia involving the susceptibility method was begun by Dr. Child in 1913. In the spring of 1916, work was begun by him on the modification and control of development in the frog, on the basis of differential susceptibility; the data and preserved material of these experiments were turned over to me late in that year. My own work was done in the spring of 1917 and the spring of 1919 during which time approximately one hundred thousand eggs were handled, of which about forty thousand were preserved.

2. *Routine and Methods of Handling Eggs.*—Clasping frogs (*Rana pipiens*) were obtained from a local dealer and placed in a large cement aquarium arranged to simulate natural conditions as nearly as possible. The temperature of the water was kept between 5° and 9° C. There was no difficulty in obtaining all the eggs that could be used; and with a little patience they could be had as soon after deposition as desired. As many as five females have been seen spawning at one time. Shortly after deposition each egg mass was given a number, the jelly cut into bits containing ten to twenty eggs each, and then placed in a gallon jar of the cold aquarium water. About an hour before being introduced into experimental conditions the jar of eggs was removed to the laboratory to warm slowly up to the room temperature (17 ± 1° C.).

For the modification of development the following chemicals were used: potassium cyanide, formaldehyde, potassium permanganate, mercuric chloride, magnesium chloride, lithium chloride, hydrochloric acid, sodium hydrate, and ethyl alcohol. The method of handling the eggs varied somewhat with the chemical used. Eggs treated with KNC, CH_2O, HCl, NaOH and C_2H_5OH, were placed in liter Erlenmeyer flasks filled almost full and stoppered. For the other chemicals, glass finger bowls of 300-c.c. capacity, one- and two-liter candy jars were used and covered with glass plates to prevent evaporation.

In a given experimental series similar glassware was used, each vessel containing approximately the same number of eggs from the same female. In every case one vessel of the series was used as a control and was treated in the same way as regards stoppering, changing of fluids, etc. Eggs from the same females developing in liter Erlenmeyer flasks filled nearly full and stoppered, and in the same volume of water in open dishes did not differ in rate or manner of development up to some time after hatching, provided the water was changed once in twenty-four hours and when there were not more than 100 c.c. of egg mass in the stoppered flask. Especial care was taken to use no more than 15 to 20 c.c. of egg mass in stoppered vessels, and to change solutions in all experiments daily.

As will be noted later, the frog egg undergoes a tremendous increase in susceptibility during the early stages of development, and to provide for this and to carry the effects of differential inhibition somewhat farther than is possible where the eggs remain in the same concentration throughout the experiment, a number of experiments were done where the solution was gradually diluted as development proceeded.

In the experiments on recovery, the eggs were removed from the solutions and washed in several changes of water to remove any of the chemical remaining in the jelly.

III. The Origin of Polarity.

1. *Polarity in Other Organisms.*—There is abundant evidence to show that for many organisms and especially among the plants, polarity and symmetry arise in response to external conditions. Polarity in the egg of the alga *Fucus*, and in the spore of *Equisetum* is usually determined by the direction of incident light. In some algæ, polarity and symmetry are directly under the influence of light, even in the vegetative thalli, and are reversible or modifiable by change in light relations. In various liverworts and free prothallia, and even in certain phanerogams, light is an important factor in determining dorso-ventrality in branches.

The polarity of a number of plant and animal eggs bears a definite relation to their manner of attachment to the parent body during growth stages. In the phanerogams, for example, the free end of the egg becomes the apical, the attached end the basal end of the plant axis, and the same relation holds for a number of the lower animals.

Likewise a polar axis of symmetry once established may be obliterated experimentally and a new one induced by conditions external to the organism. This has been done by H. V. Wilson (1907, 1911) in certain sponges and hydroids, work which was confirmed by Hargitt (1915). In experimentally produced biaxial forms in planaria, hydroids and annelids, a new axis arises 180° from the old one. In *Corymorpha*, *e.g.*, Child showed that when pieces of the stem are placed in 2 per cent. to 2 1/2 per cent. alcohol in sea water, "in the course of a few days the pieces become shorter and more rounded, decrease in size, and lose the characteristic structure of the *Corymorpha* stem." On removal to water after several days in alcohol, a new individual arises from the old tissue with its axis at right angles to the old one.

Various investigators have observed that under certain conditions, *Hydra* may lose its characteristic structure and "melt" down into a shapeless mass from which a new individual may arise. Dr. Hyman, in this laboratory, has recently observed that such masses often give rise to several hypostome regions each with tentacles.

Physiological axiation begins, of course, with the origin of polarity in the egg. Polarity may, conceivably, arise in several ways. First, axiation and polarity may, as has been commonly supposed, exist in protoplasm in relation to some intrinsic molecular or other structure which is a fundamental property of living matter; second, the primordial cell, from which the ovum arises, may inherit its polarity, which, if true, merely pushes the problem farther back; third, the appearance and position of local differences in structural and functional order in the egg protoplasm, that constitutes polarity, may arise during the growth and development of the egg in response to conditions external to it. The first alternative has been shown to be untenable, and the second offers so little satisfaction, that, in the light of the accumulated evidence, it seems necessary to consider the last alternative as the more probable one, at least until it has been shown that polarity arises in some other way.

2. *Origin of Polarity in the Frog's Egg.*—The mature egg of the frog is described as possessing a radial symmetry about an imaginary axis, the polar axis, passing approximately through the centers of the pigmented (animal, apical) hemisphere and of the unpigmented (vegetative, basal) hemisphere. This polarity is marked by the localization of most of the protoplasm in the pigmented hemisphere, by the eccentricity of the nucleus, and by the distribution of pigment. In order to determine, if possible, whether, in the frog egg, a relation exists between its polarity and conditions external to it, a study was made of the ovarian relations of the egg, especially of the relation of the polar axis to the region of attachment to the ovarian membrane and to the blood supply of the egg.

Some days or weeks before extrusion, the egg is suspended from the ovarian membrane in an epithelial sack (Fig. 1). This sack, the theca, forms at one region on the egg a narrow stalk, the pedicle, which becomes continuous with the ovarian membrane. Beneath the theca is a layer of follicular cells and beneath this, the vitelline membrane. In the theca are found the blood vessels that supply the egg.

Table I. gives the location of the pedicle with reference to the distribution of the pigment on the egg. It will be noted that in approximately eighty per cent. of the cases, the region of attachment (the pedicle) is near or on the boundary between the pigmented and unpigmented regions of the egg (Fig. 2).

TABLE I.

Location of Pedicle.	Number of Eggs Observed.	Per Cent.
Position A (see Fig. 2)	75	12.2
Position B	217	35.3
Position C	259	42.2
Position D	63	10.3

These results were obtained by taking a number of eggs at random from various parts of the ovary and determining the location of the pedicle for every egg in each sample.

A number of specimens were injected through the conus arteriosus with a lead chromate-starch-gelatin mass[1] and in the great majority of cases only those vessels lying over the unpigmented hemisphere remained uninjected and could often be traced by the color of the blood remaining in them. In several cases injected vessels were seen to pass a little distance over the unpigmented hemisphere, but in no case was an egg observed where more than a small per cent. of the vessels overlying the yolk were injected (Fig. 3). Several unsuccessful attempts were made to inject the venous system.

In order to eliminate any error due to the injection mass passing through the capillaries into the veins, several frogs were opened under normal salt solution and the circulation of the blood over the egg determined with the aid of the binocular microscope. In every case where the movement of corpuscles over the pigmented hemisphere could be seen, the circulation was away from the heart and therefore arterial. The movement of blood over the unpigmented hemisphere, where the movement of corpuscles could be seen more clearly than over the pigmented hemisphere, was always toward the heart and therefore venous. As was noted for the injected specimens, a small per cent. of the eggs showed arteries passing a little distance over the yolk. But it is especially to be noted that in every case observed, the greater part of the arterial blood supply was restricted to the pigmented hemisphere.

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[1] Guyer (2d edition, page 84). The mass was made up in a 1 per cent. uncooked starch suspension, warmed and well stirred immediately before the injection was made.

In the ovary of the frog, the polar axes of the eggs lie in every possible direction with respect to gravity, so that the possibility of polarity being determined in the ovary with respect to gravity is at once eliminated.

The data indicate that polarity in the frog egg arises at some time during the growth stages, in response to external conditions, viz., to the blood supply of the egg: that region of the oögonium chancing to be most richly supplied with arterial blood being destined to become, by virtue of this respiratory and nutritive relation, the animal pole of the egg. Naturally, the region on the surface of the egg where the capillary net work is most extensive would be effective in determining polarity rather than the point where the blood vessels enter the theca.

A region of higher oxidation rate in the egg, or at any rate the proximity of this region to a greater oxygen supply (arterial blood) is further evidenced by the appearance of pigment—a melanin (Kellicott)—over a limited surface of the egg, for it is well known that oxygen is necessary for the formation of these pigments. It is probable that the origin of polarity and the appearance of pigment in a symmetrical relation to that polarity are both expressions of the localized oxygen supply (or nutritive supply, or both) of the egg.

It may be noted here also that pigment appears most densely in the most active regions of the egg where other evidence indicates that oxidations are proceeding more rapidly than elsewhere. In other words, the density of pigmentation seems to be an expression of the rate of at least certain oxidations occurring in that region. Local increase in activity results in the formation or increase of the pigment and local decrease in activity results in diminution of pigment. Those unpigmented cells lining the archenteron in the early gastrula stages, *e.g.*, become pigmented and most densely where cell division is most rapid.[1] And to anticipate somewhat, the subjection of early gastrula stages to strongly inhibiting conditions, results in a marked diminution of pigment in the dorsal lip regions.

The data further indicate that the polar axis as well as other axes that arise later in development are primarily gradients in fundamental physiological conditions or processes, which are made evident in several ways, such as, the differential cleavage rate which results in a gradient in cell size, by gradients in susceptibility, etc. (see pp. 322–328).

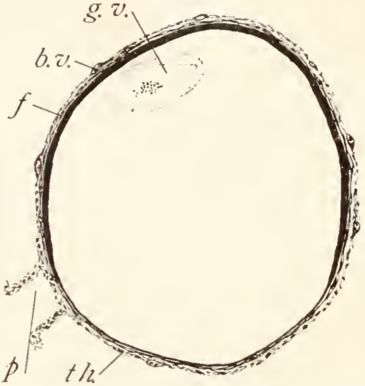

FIG. 1. Semi-diagrammatic view of a section through the germinal vesicle and pedicle of a full grown ovarian egg. *b.v.*, blood vessel in the theca; *f*, follicle cells; *g.v.*, germinal vesicle; *p*, pedicle; *th.*, theca.

Discussion of the question of whether the location of the pedicle and the localized blood supply is related in any way to the definitive bilaterality remains for future consideration.

The fact that the pedicle is equatorial in position is of course, merely a necessary consequence of the way polarity and the accompanying distribution of pigment is determined by the blood supply.

IV. EXPERIMENTAL DATA.

1. *Susceptibility to Lethal Concentrations of External Agents.*

The early stages of the frog egg are extremely resistant to toxic agents. Eggs just beginning to segment, when placed

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[1] King (1902).

e.g., in $m/10,000$ $HgCl_2$, are accelerated in development for a short time, while gastrulating eggs die within a few hours (four to six) in $m/2,000,000$ $HgCl_2$, without having developed farther after being placed in the solution. The same relation holds for other agents. Eggs from different females may differ considerably in their behavior when exposed to toxic agents; also eggs from the same female may differ somewhat in susceptibility to toxic agents but to a much less extent than in the former case. Some difficulty is experienced in watching the progress of disintegration in early stages, especially in concentrations that kill rapidly, on account of the spreading of the disintegrated protoplasm underneath the inner membrane, and thus obscuring the rest of the process. However, the progress of disintegration, wherever observed, is an orderly process and bears a definite relation to the polar axis of the egg and to the axes of symmetry of the embryo.

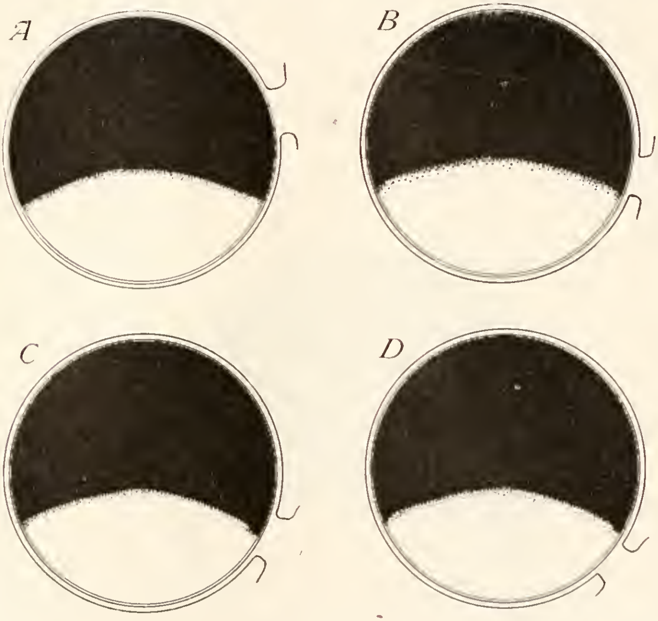


FIG. 2. Diagrams of full grown ovarian eggs to show the position of the pedicle in relation to the distribution of pigment. (See also Table I.)

(*a*) *Unsegmented Eggs.*—The most satisfactory method found for observing disintegration in the unsegmented egg, was to expose the egg soon after deposition, to a temperature of 0° C. for ten days or two weeks. Fig. 4 illustrates several different stages in the process of disintegration under such conditions. The eggs were deposited in the aquarium (temperature, 6.5° C.) March 26, 9:00–9:15 A.M., and were placed in the refrigerator at 10:00 A.M. On April 7, the eggs were in the condition shown in the figures. The relation of the disintegrated areas to the grey crescent and to the polar axis is obvious.

(*b*) *Cleavage Stages.*—The process of disintegration during early cleavage has been studied at low temperature, in KNC, $m/1,000$; $m/100$; $HgCl_2$, $m/10,000$; in alcohol, 5 per cent., 8 per cent.; and incidentally in all of the other agents used to modify development. The process of disintegration in all of these cases is essentially similar, except in the case of alcohol, which has a marked solvent action on the yolk. In any concentration of alcohol above 1 per cent., the yolk becomes rough and pitted, an appearance that is much accentuated in the higher concentrations (5 per cent. to 8 per cent.). Disintegration usually begins in a meridian that bisects the grey crescent and near the center of the pigmented hemisphere. The surface membranes of individual cells in this region break down and turn white. Shortly afterward, disintegration begins in the equatorial region just

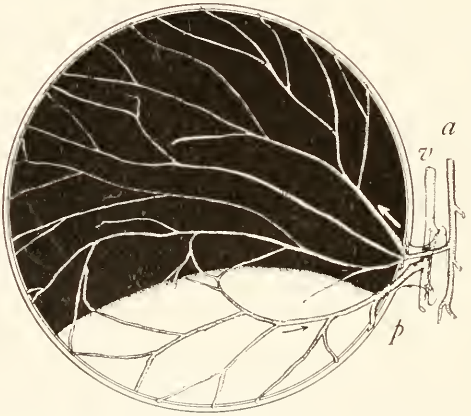


FIG. 3. Semi-diagrammatic view of ovarian egg showing the distribution of arteries and veins. The blood vessels were outlined under the camera lucida.

above the middle of the grey crescent and spreads equatorially and apically to join the area where dissolution first began. At this time the disintegrated area forms, roughly, a triangular figure whose apex is the point where disintegration first began, and whose base is the equatorial region immediately above the gray crescent. Disintegration then spreads more or less symmetrically from the apex and sides of the triangle over the rest of the animal pole, and from the base to involve the grey crescent region. Fig. 6 illustrates the process just described. The history and treatment of the eggs is given in the legends to the figures.

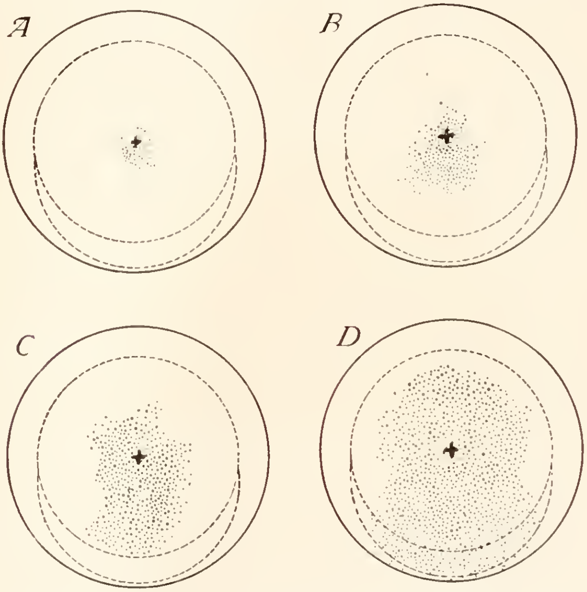


FIG. 4. Unsegmented egg seen from above (apical pole) showing different stages in disintegration after twelve days' exposure to a temperature of 0° C. Disintegrated areas are represented by stippling. The dotted circle represents the boundary between pigmented and unpigmented hemispheres. The dotted crescent outlines the gray crescent. The cross marks the animal pole.

(*c*) *Gastrula Stages.*—Eggs in an early gastrula stage, when placed in lethal concentrations of a toxic agent, always begin to disintegrate first in the dorsal lip region, and shortly afterward in the same meridian about 120° to 130° above the blastophore. From this upper point dissolution of the surface cells proceeds down the meridian and meets the disintegrated area of the dorsal lip region, which has spread apically and now includes the lateral lips. The area then spreads more or less symmetrically from the apical and lateral borders of the disintegrated area until all of the pigmented cells are involved. The yolk cells retain their structure long after the pigmented cells have completely disintegrated, except in the case of alcohol, which attacks the yolk rapidly from the start. Fig. 8 illustrates the general process.

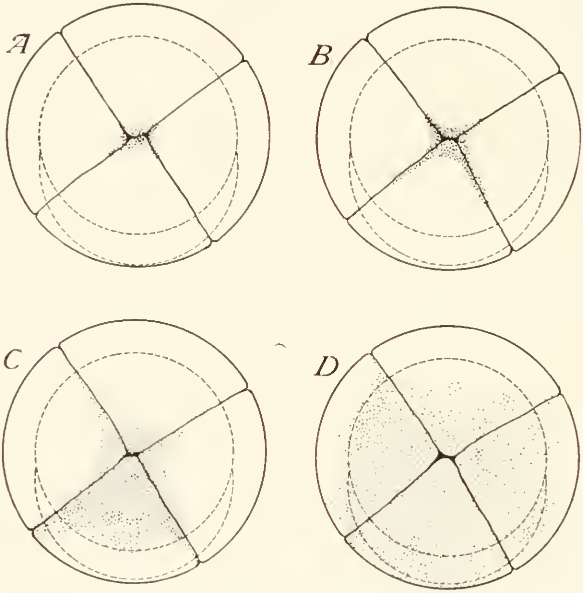


FIG. 5. View from animal pole showing stages in the disintegration of eggs in a four-cell stage after ten days' exposure to a temperature of 0° C. The eggs were in a two-cell stage when placed in the cold chamber.

(*d*) *Later Embryonic Stages.*—With the beginning of elongation, while two regions of high susceptibility are still present, viz., the apical and the dorsal lip region (now posterior), the posterior growing region, especially during the formation of the neural folds, appears relatively less susceptible than it did earlier. This apparent greater difference is probably due to local differences associated with the formation of the neural plate. In embryos beginning to elongate, disintegration begins first at the apical end in the medial dorsal region and spreads laterally and posteriorly, more rapidly in the posterior direction, to meet an area of disintegration that has just begun in the region where the tail bud appears later. In neural fold stages disintegration begins at the anterior end of the floor of the neural groove usually at two points on either side of the median line, where the primordia of the optic vesicles have appeared. Often, and especially in earlier neural fold stages, the disintegration begins in the median line at the anterior end of the neural groove. From this point or points as the case may be, disintegration spreads back along the neural groove nearly to the posterior end, where it joins an area that has just begun to undergo dissolution. In the meantime, the medullary folds, ventral suckers, and anterior part of the head region have begun to disintegrate.

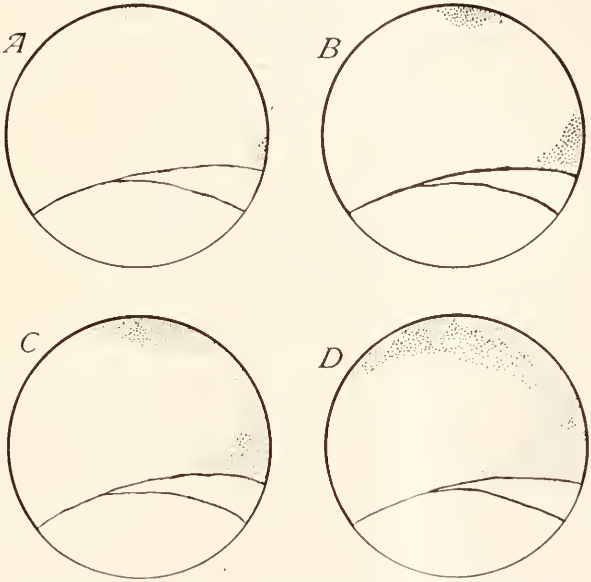


FIG. 6. Disintegration stages during late cleavage. The eggs are shown in side view with the animal pole uppermost and the gray crescent to the right.

With the closure of the neural folds and the further elongation of the embryo, local differences in susceptibility begin to appear with the differentiation of certain organs. While in general, the apical-medial-dorsal region begins to disintegrate first, the dissolution of cells in this region may be followed shortly, or even preceeded by, the disintegration of rapidly proliferating cells in certain regions of the embryo, such, *e.g.*, as the tail bud, optic vesicles, nasal pits, ventral suckers, and other rapidly growing regions.

These data on disintegration are in agreement with the data on differential inhibition, acclimation, and recovery, in showing that certain parts of the egg and embryo are more susceptible than other parts, and that these differences in susceptibility have a definite relation to the polar axis and to the plane of bilaterality in the early cleavage stages and to other physiological axes arising later in development. The significance of differential susceptibility is discussed later (pp. 346–349).

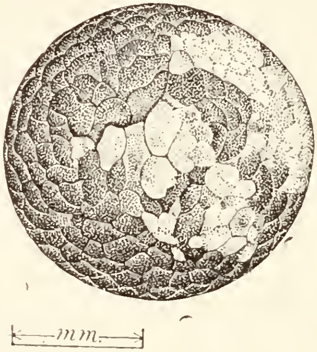


FIG. 7. Camera lucida drawing of an egg showing disintegrated cells (white) following 24 hrs. exposure to *m*/1,000 KNC and 24 hrs. to *m*/5,000 KNC, from a two-cell stage. The animal pole is in the center of the figure. (Experiment KNC B3.)

2. *Experimental Modification of Development.*

For the sake of convenience in description, certain arbitrary terms have been used to designate different "types" of abnormalities. The differential inhibition of the cleavage ratio in early development $\left(\frac{\text{size of animal pole cells}}{\text{size of vegetative pole cells}}\right)$ is expressed as a fraction whose denominator is ten. For example, the expression "cleavage = 7/10" means that the size of the animal pole cells is to the size of the vegetative pole cells, as 7 is to 10. The expression "V-shaped blastopore" refers to a condition where the blastopore takes the shape of an inverted "V" or "U," and is intended to describe conditions where the dorsal lip is retarded to a relatively greater extent than the lateral lips (Fig 14, *A*). The term "wide-crescent blastopore" refers to a condition where the blastopore is in the form of a broad crescent (Fig. 17), and is intended to describe a condition where both dorsal and lateral lips are inhibited. The term "secondary invagination" describes a situation where a second infolding takes place apical to an original equatorial blastopore (Fig. 18). The "gastrular angle" is the angle between a plane through the center of the egg parallel to the floor of the segmentation cavity, and a line through the center of the egg and the blastopore (Fig. 9). Obviously, comparison of the gastrular angle of normal eggs with this angle in inhibited eggs is possible only at the *beginning* of gastrulation.

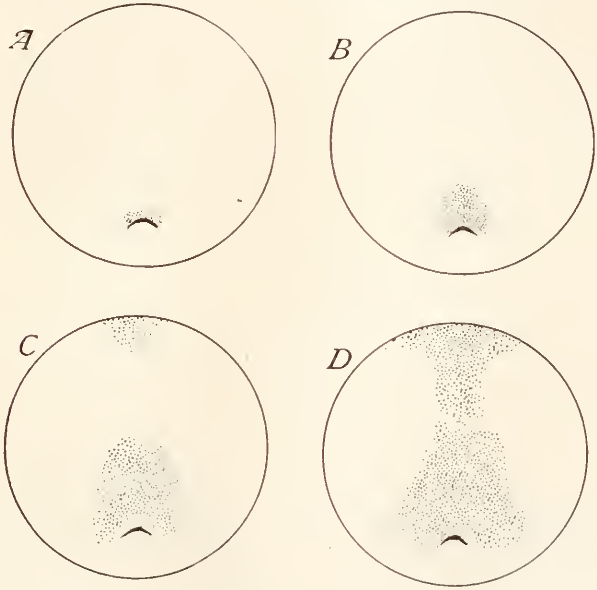


FIG. 8. Stages in the disintegration of eggs in an early gastrula stage following several hours exposure to $m/1,000$ KNC, or in $m/1,000,000$ $HgCl_2$. The progress of disintegration follows this general plan in all agents in concentrations that kill the egg within several hours.

The development of the frog is so well known that a detailed description of the process is unnecessary. However, mention of several features of normal development will be of use for comparison with abnormal types.

Cleavage begins first and proceeds more rapidly in the pigmented hemisphere than in the vegetative hemisphere, establishing early in development an inequality in the size of the cells in the two hemispheres. In an early cleavage stage, the size relation of the cells in the two regions is in the proportion of 4/10 to 5/10, a ratio that decreases in value up to about the time of gastrulation, when the ratio may be represented by 2.5/10 to 3.5/10. During mid-cleavage stages the cell size ratio is approximately 3.5/10 to 4/10. This does not take into account those pigmented cells on the grey crescent side of the egg, which are slightly smaller from the beginning of cleavage than the other pigmented cells (Morgan and Boring, 1903). Immediately preceding the appearance of the blastopore, those cells in the dorsal lip region are smaller than any of the other surface cells.

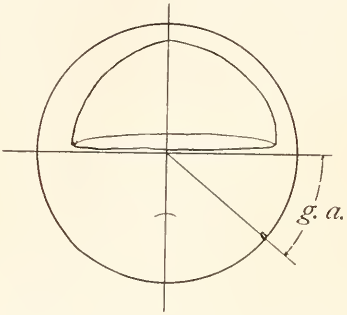


FIG. 9. Diagram to illustrate the gastrular angle, *g.a*

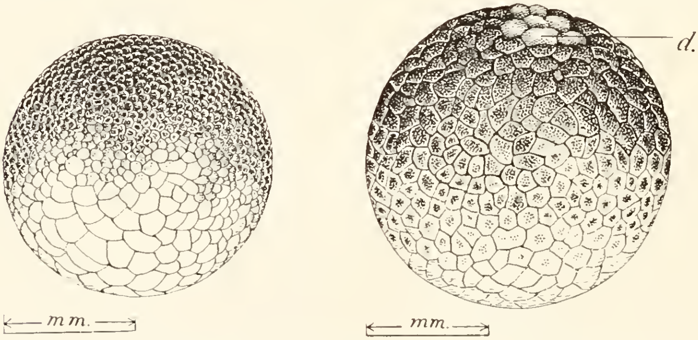
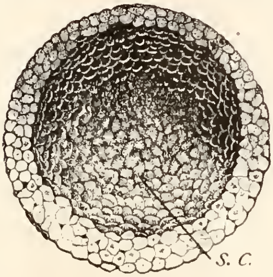


FIG. 10. *A*, control egg with a cleavage ratio of approximately 3.5/10–4/10. *B*, egg from the same batch as the one in Fig. 10, *A*, in which the cleavage ratio approximates 10/10. Same treatment as egg illustrated in Fig. 7. (Experiment KNC B 3.)

The gastrular angle at the time of the appearance of the blastopore approximates 40°.

In addition to the indications of bilaterality by the appearance of the grey crescent, bilaterality is made more apparent during the blastula stages by the movement of material from the pigmented region toward the equator—a process that takes place more rapidly in the sagittal plane than elsewhere, and results in the thinning out of the walls and roof of the segmentation cavity, which now comes to lie nearer that side of the egg where the dorsal lip appears. The so-called germ ring, formed by the downward movement of animal pole material, and which lies at first on the equator and later below it, extends somewhat farther down on the egg in the dorsal lip region. To express the situation in other words: growth in length (between the apical



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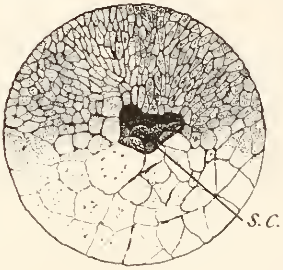

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FIG. 11. Enlarged segmentation cavity. Eggs in beginning two-cell stages treated as follows with KNC: *m*/1,000 24 hrs., *m*/5,000 24 hrs., *m*/10,000 12 hrs., *m*/20,000 12 hrs. (Experiment KNC H 5.)

FIG. 12. Small segmentation cavity as seen under conditions of severe inhibition. In this case eggs were exposed 48 hrs. from a two-cell stage to *m*/10,000 $HgCl_2$. (Experiment IV 40.)

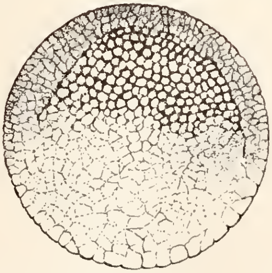

FIG. 13. Obliterated segmentation cavity. (Experiment IV 56.)

pole and the germ ring) is greater and proceeds most rapidly in the sagittal plane. The significance of this process is discussed elsewhere (p. 349).

A. *Disturbances in Cleavage such that the Egg Never Gastrulates.* (*a*) *Cell Size Ratios.*—In frog eggs so strongly inhibited that they do not gastrulate, cleavage usually begins and proceeds more or less normally for several hours. The rate of cleavage in the animal hemisphere then becomes less and less rapid, relatively, until the cell size ratio approaches and may become equal to one (10/10, Fig. 10, *B*), whereas this ratio in a normal egg of a comparable stage of development is about 3.5/10 to 4/10 (Fig. 10, *A*).

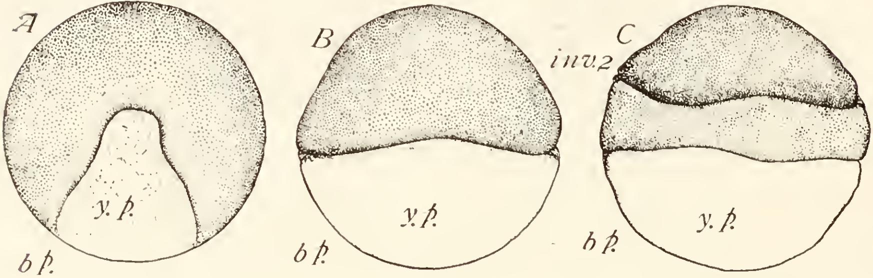

FIG. 14. V-shaped blastophore, *A*; Equatorial gastrulation, *B*; Secondary invagination, *C*. Produced in m/500,000 HgCl$_2$. About 40 per cent. of the eggs showed the V-shaped blastophore. (Experiment IV 70.)

The cleavage ratio was found to increase in value consistently in all cases[1] where inhibition was sufficient to prevent the com-

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[1] This distortion of the cleavage ratio was obtained in: Experiments KNC A 1; H 1; exposure 12 hours in m/1,000 from 2-cell stage; cl. = 5/10 to 7/10. Experiment *KNC—C.7;* exposure 24 hours in m/2,000 from 2–4-cell stage; cl. = 7/10. *Experiment KNC—C.8;* 48 hours exposure to m/2,000 from 2-cell stage; cl. = 8/10. Experiment *KNC—C.12;* 48 hours exposure to m/2,000 from 2-cell stage; cl. = 7/10 to 10/10. Experiment KNC—C 1; 12 hours exposure to m/5,000 from 2-cell stage; cl. = 5/10 to 6/10. *Experiment KNC—C.6;* 24 hours exposure from 2–4-cell stage; cl. = 5/10 to 7/10. Experiment *KNC—C.9;* 5 days exposure to m/10,000 from 2 cell-stage; eggs beginning gastrulation (equatorial) with 6/10 to 8/10 cl. Experiment IV 21; 21 1/2 hours exposure to m/8.5 LiCl from an unsegmented stage; in about 15 per cent. to 20 per cent. cl. = 4/10–7/10. Experiment IV 31; unsegmented eggs exposed 4 hours in m/4 LiCl, then 7 1/2 hours in water showed an 8/10 to 10/10 cleavage ratio. Experiment IV 54; eggs in late cleavage exposed 40 hours to m/100,000 NgCl$_2$; cl. = 6/10 to 10/10. Experiment IV 62; eggs in 4–8-cell stage exposed 12 hours in 0.0075 per cent. formaldehyde; cl. = 6/10 to 10/10. Citations of experiments in italics are from Dr. Child's preserved material. "cl." = cleavage ratio.

pletion of gastrulation, although the proportion of eggs showing this type of differential inhibition varied somewhat in experiments involving different chemicals. This increase in value of the cleavage ratio appears less frequently in LiCl, $MgCl_2$, and in alcohol, and most frequently in KNC and formaldehyde. In $m/1,000$, $m/2,000$ KNC, fully 90 per cent. of the eggs are of this type after 24 to 36 hours' exposure from the beginning of segmentation. Differential inhibition under the conditions stated, is diagrammatic, especially in KNC, and represents simply a greater susceptibility to the toxic agent, of the cells most affected, viz., the animal pole cells. The significance of this differential susceptibility is a matter of discussion elsewhere (pp. 346–349).

(*b*) *Bilaterality.*—Under the conditions of these experiments, bilaterality is much obscured and may not become evident at all (Fig. 20). But, that bilaterality is at least potentially present is indicated in those cases where gastrulation does begin (always equatorially) the wrinkling and folding, indicating the initiation of gastrulation usually begins at a definite point on the equatorial region and spreads equatorially around the egg. A few cases have been observed in $m/5,000$ KNC where the equatorial folding occurred almost simultaneously around the entire equator of the egg.

(*c*) *The Segmentation Cavity.*—Under severe inhibiting conditions, the segmentation cavity shows several characteristic consequences of differential inhibition, depending chiefly upon the severity of the conditions and the stage at which the eggs are exposed to them and upon the length of the exposure.

Some of the data are these: In eggs exposed to $m/1,000$ or $m/5,000$ KNC (Experiments KNC A–H) from the beginning of the first segmentation, development usually ceases in late cleavage stages with occasional abortive attempts at equatorial gastrulation. The yolk cells, which are relatively less inhibited, may continue division for a time after it has nearly or quite stopped in the animal hemisphere. In such cases, one finds that the more peripheral yolk cells, forming the floor of the segmentation cavity, proliferate apicalward, forming a thin layer of yolk cells that may partly or completely line the walls of the segmentation cavity—which becomes very large. At the time of death, the egg resembles in some respects an amphioxus blastula (Fig. 11). Superficial indications of bilaterality are obscure.

Where inhibition is more severe, *e.g.*, in *m*/10,000 $HgCl_2$ (Experiment IV 40), eggs exposed to the solution for 48 hours from a two-cell stage, stop development in late cleavage stages. Gastrulation never occurs. In all of the eggs, the downward migration of materials from the apical pole is completely inhibited and the walls of the very small segmentation cavity lying near the center of the egg, are 2/5 to 4/9 the diameter of the egg in thickness (Fig. 12).

The partial or complete obliteration of the segmentation cavity may occur in an entirely different way under less severe inhibiting conditions. Eggs in late segmentation stages, exposed 15 minutes to *m*/10,000 $HgCl_2$ (Experiment IV 56) then returned to water, show after three days, the segmentation cavity completely filled with small yolk cells that have proliferated from the floor of the cavity. A faint line indicates the walls of the now obliterated cavity. The walls of what was the segmentation cavity are much thinner than when the eggs had been exposed 48 hours to *m*/10,000 $HgCl_2$ from the beginning of cleavage.

Likewise in *m*/10 LiCl, where development proceeds abnormally to the time of hatching, the proliferation of yolk cells from the margin of the floor of the segmentation cavity may be so extensive as to fill it completely.

All of these different modifications appear to be simply different expressions of the differential inhibition which the eggs have suffered. The animal pole cells being most susceptible, are most affected by the adverse conditions, the yolk cells least.

Those small cells around the margins of the floor of the segmentation cavity, which under certain conditions of inhibition, continue division and finally fill the blastocoele completely, appear to be the ones destined to give rise to mesoderm (Morgan, 1906, p. 129; Kellicott, 1913, p. 107; King, 1902, Fig. 4). It is significant here that in the sea urchin Child (1916, p. 91) found the mesenchyme cells which arise from the basal pole of the egg, to be less susceptible than other parts of the egg, and that under conditions of differential inhibition, these cells tended to "run wild" and result in an excessive over-development of skeletal structures.

(*d*) *Meroblastic Cleavage.*—In exceptional cases, and under the influence of conditions so severe that development stops completely in early cleavage, cell division may be partly or completely restricted to the animal pole. This type of cleavage has been observed following exposure: to urea, 2.34 per cent. (Jenkinson, 1906, Figs. 38, 41); to NH_4I (Jenkinson, 1906, Fig. 32); to Na_2SO_4 (Jenkinson, 1906, Fig. 40); to temperatures above 26° C. (Hertwig, 1895), et al. In my own experiments it has been observed among eggs exposed six to ten hours to 2 per cent., 3 per cent. alcohol, a result that is not surprising when one considers the solvent action of alcohol upon lipoid substances generally. Four factors seem primarily concerned in the production of these restricted cleavages, viz., time, concentration or intensity of action of the agent used, physical effect of the particular agent to which the eggs are exposed, and the stage at which the eggs are exposed to the agent. So far as my observations go, this type of cleavage, rare at best, is realized only when the eggs are exposed to the inhibiting conditions at the time of, or immediately preceding the appearance of the first cleavage plane. If the eggs are exposed to concentrations necessary to the production of this type of cleavage, several hours before cleavage would normally begin, they never segment. The physical effect of alcohol has been mentioned. The time element is more or less obvious. Cleavage begins first, *normally*, in the pigmented hemisphere, and under the conditions necessary for the production of meroblastic cleavage the first cleavage plane makes its appearance, in all probability, before the effect of the inhibiting conditions (in the case of chemical substances particularly) penetrate the gelatinous membranes of the egg.

B. *Disturbances in Gastrulation.*—The various modifications of the process of gastrulation, following inhibition, fall into several more or less distinct groups, the particular types obtained depending largely upon the severity of the inhibiting conditions and upon the stage at which the eggs are introduced into the experiment; *i.e.*, depending upon the treatment and the physiological condition of the eggs. Individual variation is an important limiting factor in the experimental control of modifications. The range of variation in this respect seems to be widest during the early cleavage stages. In the great majority of experiments, while one usually finds a complete series of stages from nearly normal to the most extreme deviations from the normal, the modifications produced tend to conform to certain types, varying with the physiological condition of the eggs and the treatment they receive. It is therefore necessary to speak in terms of averages in discussing a given type of abnormality.

In general, as the concentration of a given chemical to which the eggs are exposed, is increased, or with longer exposure to the same concentrations, the modifications produced become more extreme. They are discussed in the order in which they appear.

(*a*) *Retardation of the Dorsal Lip Region.*—These are stages in which only the dorsal lip region seems much inhibited, although occasionally the cleavage ratio may increase in value somewhat, and the gastrular angle (see p. 329) in a small per cent. of the cases may be slightly more acute than in the control eggs. Where only the dorsal lip region is inhibited, the blastopore takes the form of an inverted V or U and when complete the yolk plug is oval, and may be several times longer than broad in the sagittal plane.

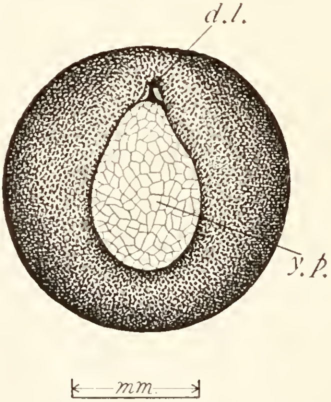

FIG. 15. Inhibition of the dorsal lip region after 38 hours exposure to $m/10.62$ LiCl. Eggs placed in the solution were in 16–32-cell stages—24 hours after deposition. (Experiment IV 76.) *d.l.*, dorsal lip region; *y.p.*, yolk plug.

Experiments IV 70; G, H, I. Eggs deposited March 28, 3:00 P.M. Removed to the laboratory 4:00 P.M. Introduced into $m/500{,}000$ $HgCl_2$, 5:00 P.M.—unsegmented at this time. After 24 hours the eggs were washed and returned to water. Twenty-four hours later (March 29, 5:00 P.M.) the stages shown in Fig. 14 were recorded. About 40 per cent. of the eggs showed the V-shaped blastopore (Fig. 14, *A*). Eggs in the three experiments were alike and among them everything was present from a few with closed blastopores to several forms showing a secondary invagination midway between the original (equatorial) blastopore and the apical pole. The equatorial gastrulæ resemble those produced in $m/10$ LiCl.

Experiment IV 91. Unsegmented eggs, placed in $m/2{,}000{,}000$ $HgCl_2$. Forty hours later, the eggs were mostly in inhibited gastrula stages (V-shaped blastopores).

Eggs introduced into $m/10.62$ LiCl during 16–32 cell stage, exhibit the inverted V- or U-shaped blastopore after 24 hours' exposure (Experiment IV 76). Eggs introduced into $m/10$ LiCl immediately before gastrulation (Experiment IV 59) are in gastrula and large yolk plug stages after eighteen hours in the solution. The majority of gastrula stages show the long inverted V-shaped blastopore and in those eggs showing yolk plugs, there is a tendency for the yolk plugs to be much larger than normal and somewhat oval in shape (Fig. 16).

After 48 hours in the solution, ninety per cent. of the eggs were in early neural fold stages, with much elongated yolk plugs.

In experiment IV 62 unsegmented eggs, five hours after deposition, were introduced into 0.0075 per cent. formaldehyde, and varied from equatorial gastrulæ to early neural fold stages after sixty hours' exposure. A few showed secondary invaginations.

In experiment R 138 a, eggs in early gastrula stages were placed in $m/1{,}000$ KNC. After a 12-hour exposure, 90 per cent. of the eggs showed a long inverted V-shaped blastopore or much elongated yolk plug. The eggs did not develop farther in this solution.

(*b*) and (*c*) *Retardation of Both Dorsal and Lateral Lips of the Blastopore, and Equatorial Gastrulation.* Eggs showing retardation of both dorsal and lateral lips are described as "flat-crescent" gastrulæ, for the reason that under the slightly more severe inhibiting conditions where both dorsal and lateral lips are inhibited, the blastopore forms, when about half completed, a figure like a much flattened crescent. When such a blastopore is complete the yolk plug is always much larger than in the control. All stages exist between this flat crescent type and those cases where the blastopore extends around the equator of the egg (equatorial gastrulation). In these forms the cleavage ratio

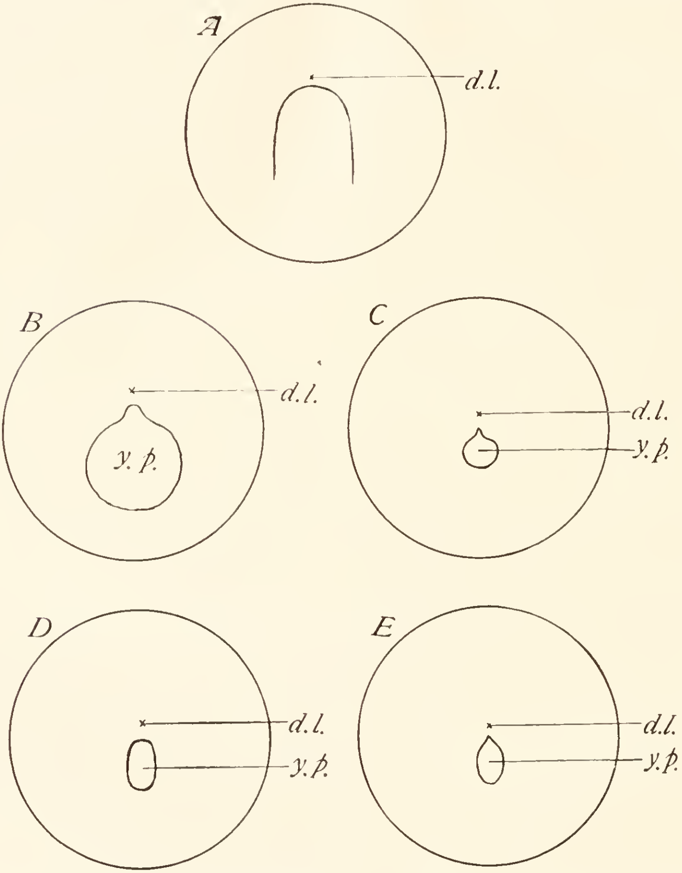

FIG. 16. Inhibition of dorsal lip region. Eggs placed in $m/10$ LiCl in late cleavage stages immediately before gastrulation. *A* and *B* after 18 hrs. in the solution; *C*, *D* and *E* after 54 hrs. in the solution. *d.l.*, dorsal lip region; *y.p.*, yolk plug. (Experiment IV 76.)

varies from nearly normal (in the less inhibited ones) to 10/10 (more inhibited ones). In the more extreme inhibitions some cases of secondary invaginations are seen and a decrease in the gastrular angle is characteristic. Almost any type may be produced at will simply by varying the concentration of the inhibiting agent, or the length of the exposure, or the stage at which the egg is introduced into the experiment, or by varying several of the factors at once. The chief limiting factor in the control of these and other modifications is the variation in susceptibility exhibited by the eggs of different females. Several preliminary attempts may be necessary before the desired result is obtained. Some of the experiments are cited below, in which these modifications were prominent, and which serve to illustrate a few of the many different ways in which these abnormalities may be produced.

Experiment IV 21.[1] Unsegmented eggs, introduced into $m/7$ LiCl 1 1/2 hours after fertilization. After 21 hours in LiCl and 7 hours in water a few of the eggs showed a slight equatorial wrinkling but no distinct blastopore was present. After 24 hours in LiCl, and 24 hours in water most of the eggs were in late blastula stages in which the segmentation cavity was completely lined with one or more layers of small round slightly pigmented cells.

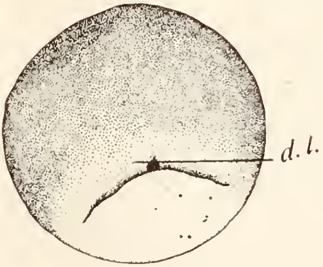

FIG. 17. Inhibition of both dorsal and lateral lip regions of the blastophore, resulting in a "flat-crescent" blastopore. 28 1/2 hours' exposure to $m/10.62$ LiCl from unsegmented stage. (Exp. IV 22.)

Experiment IV 21. After 20 hours in $m/8.5$ LiCl and 8 hours in water, about 80 per cent. of the eggs were beginning equatorial gastrulation. After 20 hours in this solution and 22 hours in water, about 70 per cent. of the eggs showed various stages of equatorial gastrulation; the rest of the eggs varied from nearly normal yolk plug stages to those showing flat-crescent blastopores (Fig. 17). After 76 hours in water following 20 hours' exposure to the solution about 5 per cent. of the eggs showed a secondary invagination apical to the equatorial blastopore. This secondary invagination appeared more prominently (in about 40 per cent. to 50 per cent.) after 28 hours in $m/8.5$ LiCl and 48 hours in water (Fig. 18).

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[1] Eggs used in experiments IV 21, 22, 26, 27, 29, 31, 33, 34, all came from the same female, and were introduced into the different experiments 1 1/2 hours after deposition.

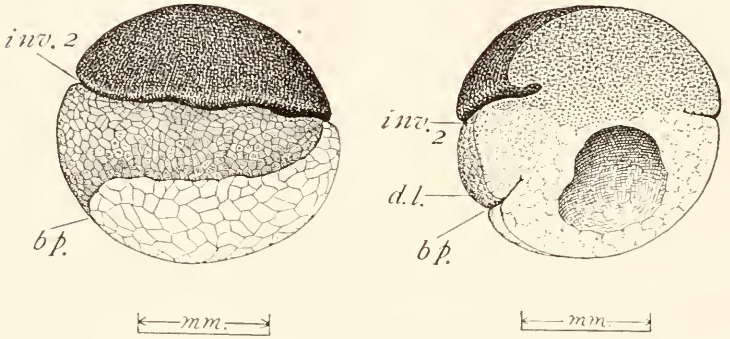

FIG. 18. Secondary invagination following 48 hours' exposure to $m/10$ LiCl, from an unsegmented stage. *A*, surface view; *B*, sagittal section of same egg. *inv.* 2, secondary invagination; *d.l.*, dorsal lip; *bp.* blastopore; *s.c.*, segmentation cavity. (Exp. LiCl 1 d.)

Experiment IV 22. 48 hours' exposure to $m/10.62$ LiCl gave 90 per cent. equatorial gastrulæ (Fig. 19), which when returned to water proceeded to develop farther and after 28 hours were in elongated neural fold stages with large protruding yolk plugs. After 72 hours in water following 48 hours' exposure to the solution one finds all stages from a few equatorial gastrulæ to microcephalic and anencephalic forms with partially or completely "fused" suckers, nasal pits, and optic vesicles. After 76 hours' exposure to the solution and 20 hours in water the abnormalities were more extreme. In about 5 per cent. of these the pigmented cells grew out from one to ten mm. to form a cone of cells radially symmetrical about the original polar axis of the egg, there being not the slightest external evidence of bilateriality or dorsoventrality (Fig. 20).

Unsegmented eggs exposed 48 hours to *m*/1,000,000 $HgCl_2$ exhibited all stages between equatorial gastrulation and fairly normal yolk plug stages. Eggs with a broad crescentic blastopore were most prominent. The eggs died without developing farther.

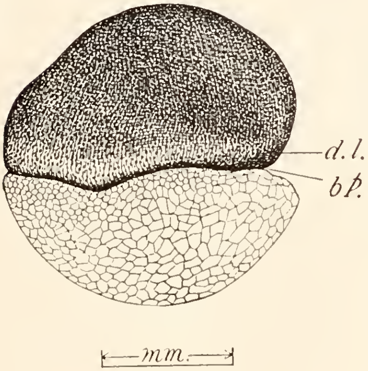

FIG. 19.

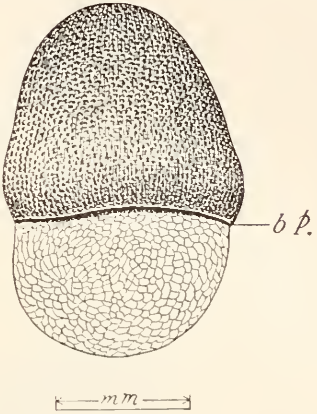

FIG. 20.

FIG. 19. Equatorial gastrulation following 76 hours' exposure to *m*/10.62 LiCl from an unsegmented stage. (Experiment IV 22.3.)

FIG. 20. Embryo radially symmetrical about the polar axis. Produced by exposing unsegmented eggs 76 hrs. to *m*/10.62 LiCl; 20 hrs. in water. (Experiment IV 22 b.)

Experiment IV 34. Eggs in 8-cell stage (10 hours and 20 minutes after deposition) were placed in *m*/5,000 $KMnO_4$. After 48 hours' exposure—solution not changed—the eggs showed a complete series of stages from perfect equatorial gastrulæ to normal yolk plug stages. At this time all of the permanganate had been reduced to the brown form and the solution had evidently lost much of its toxicity. Sixty hours after being placed in the solution about 50 per cent. of the embryos were elongating but were markedly macrocephalic, showing differential recovery.

(*d*) *Secondary Invaginations.*—The eggs described by this term show a secondary invagination appearing in a meridian bisecting the original blastopore which is usually equatorial. The invagination may extend partly or completely around the egg and appears only under conditions of severe inhibition. In addition to this secondary inturning, some overgrowth may take place; in some cases the over growth is in the direction of the vegetal pole as in normal gastrulation, while in other cases the overgrowth is toward the apical pole. In eggs where this upward overgrowth is taking place over the secondary invagination, the yolk cells below the original blastopore are usually overgrowing the pigmented cells of the animal hemisphere, thus reversing the usual process.

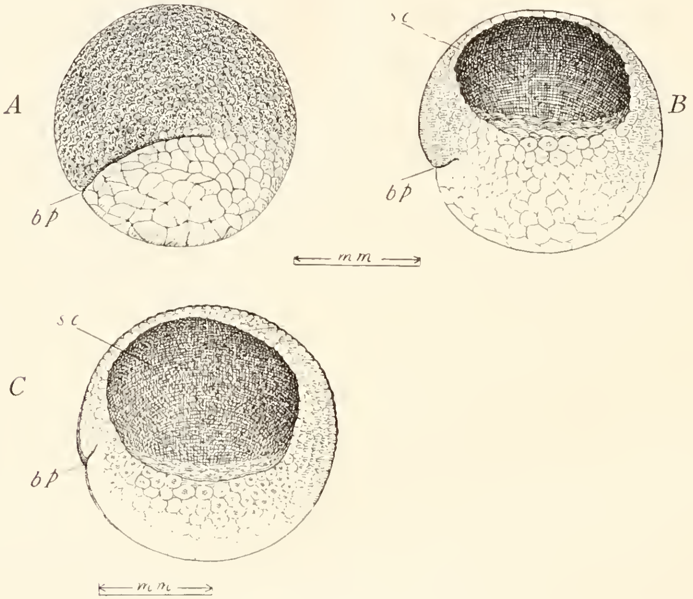

FIG. 21. Some types of modification seen after the following treatment in KNC: *A*, *B* (same egg). Eggs beginning two-cell at start of experiment; 24 hrs. in *m*/1,000, 24 hrs. in *m*/20,000, 12 hrs. in *m*/50,000, 12 hrs. in *m*/100,000. C, 12 hrs. *m*/1,000, 12 hrs. *m*/5,000, 24 hrs. *m*/10,000. (Experiment KNC A 3.) Note the position of the blastopore which is nearer the equator than in control eggs.

This modification is similar to that described by Morgan (1903), which he says is produced by a process in which "the cells of the upper hemisphere . . . turn into the egg." It makes little difference whether one refers to the process thus or whether one calls it simply an invagination. I have used the latter term because the process appears to me to be similar in many respects to that of invagination and overgrowth seen in gastrulation.

Some of the experiments in which this secondary invagination appeared have been cited. They are illustrated in Figs. 14, *C*, and 18. Several other experiments follow.

Experiment LiCl 1. Unsegmented eggs placed in $m/10$. 6 hours: not appreciably different from control. 24 hours: beginning equatorial gastrulation. Pigment has decreased somewhat in the cells of the dorsal lip region. The probable significance of this disappearance of pigment has been mentioned (p. 321). 30 hours: large yolk plug—equatorial gastrulæ. 48 hours: equatorial gastrulæ with secondary invaginations.

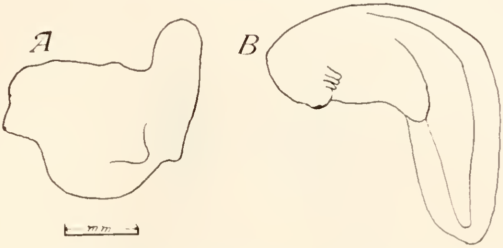

FIG. 22. *A*, differential inhibition—fused suckers, nasal pits, dorsal concavity. Three hours' exposure (from late cleavage) to $m/5$ LiCl; four days in water. (Experiment IV 58 b.) *v.s.*, ventral sucker; *n.p.*, nasal pit. *B*, differetnial recovery. Note the marked dorsal convexity. (Experiment IV 59 e.) Two days' exposure (from late cleavage) to $m/10$ LiCl; five days in water.

In experiment IV 70 (see p. 337) this modification occurred in about 10 per cent. of the eggs following 24 hours' exposure to $m/500,000$ $HgCl_2$ and 24 hours in water. A few cases were noted following 48 hours' exposure, from unsegmented, to 0.0075 per cent. formaldehyde (Experiment IV 83). In experiment R 135, eggs just beginning to gastrulate were placed in 0.001 per cent. formaldehyde and after 24 hours' exposure showed equatorial gastrulæ with secondary invaginations similar to the one shown in Fig. 18. Eggs just beginning to segment when subjected to the following graded treatment showed various degrees of this modification in about 50 per cent. of the eggs: KNC $m/1,000$ 24 hours, $m/20,000$ 24 hours, $m/50,000$ 12 hours, $m/100,000$ 12 hours. (Experiment KNC H 5).

(*e*) *The Gastrular Angle.*—In the normal egg the blastopore normally makes its appearance in a meridian that bisects the grey crescent and about 40° below a plane through the center of the egg and parallel to the floor of the segmentation cavity. It is characteristic of strongly inhibited eggs that the gastrular angle approaches and often equals 0. Not infrequently the angle becomes negative, *i.e.*, a circular blastopore is formed *above* the equator. Some of the experiments where this shifting of the point of appearance of the blastopore occurred have been cited (pp. 337, 339, 340). Several variations of this modification are illustrated in Fig. 21.

Under conditions where marked acceleration of development occurs, *e.g.*, in *m*/100,000 KNC; *n*/5,000 to *n*/20,000 HCl[6] (2 c.c. to 5 c.c. of *n*/10 HCl in well water) made up in well water, there is a tendency for the gastrular angle to approximate 45° or even 50°. It is much more difficult to alter the space relation of the blastopore and apical region by acceleration than by inhibition. It may be noted that the effects of acceleration become more apparent about the time of hatching. In these cases the embryos are markedly macrocephalic and dorsally convex, indicating more rapid growth of the anterior and dorsal parts.

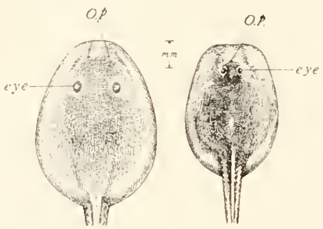

FIG. 23. *A*, normal. *B*, microcephalic, eyes closer together than normal, nasal pits fused, ventral suckers absent. Three hours' exposure (from early gastrula) to *m*/7 LiCl; 13 days in water. (Experiment R 122.1.)

C. *Differential Inhibition in Later Development.*—The whole problem of differential susceptibility involves of course, not only differential inhibition, with which this paper is primarily concerned, but it also includes differential acclimation and recovery, the data on which are fully as striking and significant as those on differential inhibition. But their consideration is reserved for future consideration along with a detailed discussion of differential susceptibility in the later stages of development in the frog's egg. However, for the sake of completeness a few of the more striking abnormalities of later development may be mentioned. These are: microcephalic forms with such bilateral structures as optic vesicles, ventral suckers, and nasal pits in all degrees of approximation from nearly normal to complete "fusion."[1] These forms are seen somewhat more frequently in $m/10$ LiCl than in other agents but they have been obtained in varying percentages in all of the agents used to modify development. Several of the forms are illustrated in Figs. 22–25. The history and treatment of the eggs is given in the legends to the figures.

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[6] I am aware that acid is generally regarded as an inhibitor of protoplasmic activity, but in these experiments the acceleration of development in certain concentrations of HCl is perfectly definite and distinct. Similar results were obtained independently and repeatedly by both Dr. Child and myself. Since the well water in which the solutions were made up contains various salts and other substances in minute amounts it remains to be decided whether the acceleration of development in these cases is a direct or an indirect effect of the acid.

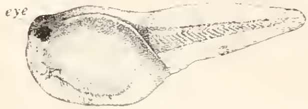

FIG. 24. Cyclopic frog embryo. (Experiment R 122.1.)

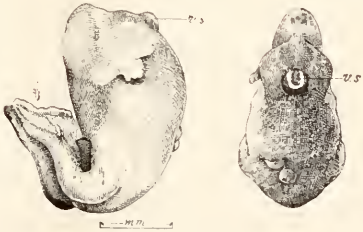

FIG. 25. Spina bifida, microcephalic, ventral suckers nearly fused. Twelve hours' exposure (from early gastrula) to $n/300$ NaOH; four days in water. (Experiment R 125.4.)

In addition to the marked apical inhibitions just mentioned the later stages show also considerable median dorsal inhibition

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[1] The term "fusion" is used in a purely descriptive sense for the eye of cyclopic forms, single median nasal pits, or single median ventral suckers. Whether the primordia of the organs mentioned arise as paired structures and later fuse to form a single median structure, or whether a single primordium arises that later divides into two parts in normal individuals, but which do not separate under strongly inhibiting conditions, is a question for future consideration.

(Fig. 22, *A*) indicated by the marked dorsal convexity with the tail extending upward, in some cases at right angles to the body. The medullary folds may fail to close in the brain region, in the brain region and at the posterior end, or they may not close at all throughout their entire length. In some cases anencephalic forms appear. Spina bifida of all degrees is of course common under conditions that inhibit development, and result primarily from inhibition of the dorsal lip region.

V. Discussion.

It has been shown that gradients in susceptibility to several external agents do exist in the egg and embryo of frog. Concerning the *fact* there can be no question. It was pointed out that the experimentally produced modifications described in this paper are readily and logically accounted for on the basis of differential susceptibility. Now, since no evidence was found of: (*a*) "specificity" in the sense in which the term has been used to explain terata produced by various chemical agents; (*b*) of "blastolysis" or (*c*) of any "disorganization" effect of the chemicals used to modify development as effective factors in the production of terata, the interpretation of the data is now largely a matter of pointing out the basis and significance of differential susceptibility. This is the chief task of the discussion.

1. *The Nature of Physiological Axes.*—These differences in susceptibility to external agents exhibited by different parts of the living egg and embryo (see pp. 322–344) must represent differences of some sort in protoplasmic conditions. Living protoplasm is a complex system of correlated, interdependent reactions and conditions, and it is unquestionably true that different agents affect it differently or attack it at different points. But, whatever the particular factor or factors chiefly concerned in determining these differences in susceptibility to particular agents may be, whether it be rate of fundamental oxidation-reduction processes, state of colloidal aggregation, permeability of limiting membranes, or something else, the general uniformity and lack of specificity in the susceptibility relations to different agents and conditions, not only as regards the egg and embryo of the frog but as regards animals and plants generally, force us to

certain conclusions. These are briefly: (1) that differences in susceptibility must depend primarily upon quantitative differences in protoplasmic condition, rather than upon the specific or qualitative differences, and (2), that these quantitative differences are closely associated with differences in the rate at which fundamental physico-chemical processes are taking place in different regions of an individual or in different indivduals.

These facts, viz., differential susceptibility relations and their marked uniformity and lack of specificity, not only in the frog but also in other organisms; gradients in protoplasm-yolk and pigment distribution both of which parallel the polar axis; and the fact that development begins and proceeds in a definite, orderly, and sequential manner with respect to certain axes (or to the polar axis) in such a way that structural and functional order are at all times referable to one or more axes or planes of symmetry (anterior-posterior, dorso-ventral, medio-lateral), constitute evidence for the existence of a fundamental underlying order of some sort that exists prior to, and which conditions the orderly, sequential, and spatial relations of parts that become apparent later in development. Furthermore it is this order, of which differential susceptibility is one expression, which affords the basis for the great uniformity and lack of specificity in the susceptibility relations, not only in the frog but also in other organisms.

The nature and origin of this underlying order is the problem of physiological axiation—a problem that has been the subject of no little speculation and experimentation. It has been thought by some that polarity and symmetry as manifested in the organism are fundamental properties of protoplasm, and, like the polarity and symmetry of crystalline substances or of the magnet, are intra-molecular in character. It may be said, however, that this conception and others of a similar nature, do not stand the test of experiment even where capable of such an analysis.

On the basis of extensive experimental work Child has arrived at a dynamic conception of physiological axes in the organism with the aid of which he accounts in a satisfactory manner not only for many of the hitherto unrelated facts of normal and abnormal development, but also he has been able experimentally to modify and control development on a predictable basis.[1] Child looks upon a physiological axis in its simplest terms as a quantitative gradation in physiological condition. He has brought forward evidence from a number of sources which point to the conclusion that this gradient is a gradation in rate of metabolism, or at least of certain fundamental reactions such as the oxidation-reduction processes, which is associated with a gradation in the physical condition and constitution of the protoplasm.

If a gradient in physiological condition is a fundamental ordering factor in the process of physiological axiation, it should be possible to obtain as many expressions of this gradient as there are aspects into which fundamental metabolic activity and the physical constitution of the protoplasm may be analyzed. Hence, we might expect to find, *e.g.*, gradients in irritability; in growth and differentiation and development; in the visible morphological condition of the protoplasm; in permeability; in the active mass of enzymes; in electrical potential; in oxidation as measured by oxygen consumption and in carbon dioxide production; and conversely, gradients in susceptibility to lack of oxygen. Well known biological facts afford demonstrative evidence for the existence of some of these gradients. Further evidence for the existence of other of these gradients has been published by Child, Hyman, et al.[1] Other evidence is yet unpublished.

"All the various lines of evidence considered agree in showing that axial gradients in the dynamic processes are characteristic features of organisms and that a definite relation exists in each individual between the direction of the gradient in any axis and the physiological and structural order which arises along that axis. In the major axis the region of highest rate in the metabolic gradient becomes the apical or anterior region of the individual, and in the minor axes also the regions of highest rate in the gradients represent particular features of the order in each case. Along any axis particular parts apparently represent particular levels in the gradient. The variety, extent, and agreement of the evidence is all the more interesting in view of the fact that such gradients have not heretofore been recognized as characteristic features of organic constitution." (Child, 1915*a*, p. 87.)

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[1] See footnote 1, page 315.

2. *Origin of the Dorsal Lip Region.*—The data on differential killing and differential inhibition in the frog show, not only that the apical pole region is more susceptible than other parts of the egg during early development, but also that as development proceeds a secondary region of high susceptibility appears in the sagittal plane about 100° below the apical pole. This is the dorsal lip region. Morgan and Boring (1903) showed that in the eight cell stage, that cell of the upper quartet lying nearest the grey crescent is slightly smaller than the other three cells of the quartet. This inequality persists up to a stage some time after the beginning of gastrulation, cell division being somewhat more rapid here than elsewhere on the egg. It is well known that during early cleavage some of the apical cells migrate or are pushed downward to form a so-called germ ring that lies at first on the equator of the egg and later below it. Correlated with the greater activity of the apical pole cells in the sagittal plane, this migration or pushing is most rapid in this plane, *i.e.*, in that pigmented quadrant the median meridian of which bisects the grey crescent. This displacement of material from the apical pole region is probably not to be conceived as an active migration but as a consequence of the more active cell division and growth in the embryonic meridian. After the materials in this meridian have been displaced a certain distance from the apical pole they seem to get beyond the range of correlation of that region; certainly they become more active and establish the posterior growing region (dorsal lip). At the time of gastrulation some of the material included in the dorsal lip cells is certainly not less than 100° from where it was in the four-cell stage. What this migration of the materials of the apical region toward the equator of the egg amounts to, of course, is a growth in length which is greatest and proceeds most rapidly in the sagittal plane.

This dorsal lip region, which arises secondarily as a rapidly growing posterior region, appears normally at a fixed distance (about 130°) from the apical end of the polar axis, and it is shown that this distance decreases with increasing inhibition (decrease in metabolic rate) of the apical region, and increases slightly with increase in activity (acceleration) of the apical region. The facts suggest at once a definite physiological correlation between the apical region and the region of the dorsal lip which arises secondarily as a posterior region of active growth and differentiation, and which does not appear to differ fundamentally from the posterior growing region which gives rise to the trunk in annelid larvæ (Child, 1917*a*).

From these facts, viz., the secondary origin of the dorsal lip region, its definite spatial relation to the apical region, which may be controlled experimentally, and its appearance at the distal end of a rapidly growing region, the origin of the dorsal lip region offers an interesting parallel to those cases in certain lower animals and plants where new zoöids or new individuals arising by asexual reproduction have been shown to appear as physiological isolations.[1]

In the simple cases of physiological isolation in lower animals such, *e.g.*, as the flatworms, it appears that as the body increases in length the posterior region comes to lie beyond the range of dominance (control) of the apical end. As a consequence, the posterior end undergoes much the same changes that it would if physically isolated. It begins to dedifferentiate and its susceptibility increases, *i.e.*, it comes to resemble physiologically younger tissue. If these processes go far enough the posterior region gives rise to a new zoöid which in time may become a new individual.[1]

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[1] The rôle of physiological isolation, dominance and subordination in development has been studied by Child and fully stated by him (1915*a*, pp. 88–169) and need not be considered here except to mention some of the pertinent facts. In Child's own words:

"The experimental evidence demonstrate, first the essential independence of the apical region in both plants and animals, and secondly, determination and control by this apical region of the developmental processes at other levels of the major axis of the individual." (1915*a*, p. 125.)

The facts indicate for those forms investigated, that the control of subordinate parts by the dominant region is transmissive rather than transportative in character, and that the range of dominance, *i.e.*, the distance over which the control of the dominant region is effective and beyond which physiological isolation occurs, varies with the relative metabolic rates of the dominant and subordinate parts, and condition of the conducting path; increasing with increase in metabolic rate in the dominant regions and with the specialization of the conducting path, and decreasing with decrease in the metabolic rate of the dominant region, with increase in rate of the subordinate region, and with interference with the conducting path. These four ways in which physiological isolation may occur are summed up by Child, thus:

"It is possible to control and alter experimentally the spatial relations of parts in the individual by altering the length of the metabolic gradient and so the range of dominance. Parts of the individual may come to lie beyond the range of dominance in consequence of increase in size of the whole, of decrease in range and degree of dominance by decrease in the metabolic rate in the dominant region, of decrease in conductivity of the paths of correlation, and of the direct local action of external factors which increase the independence of subordinate parts." (1915*a*, p. 169.)

In the annelids such a region arises early in development but instead of producing zoöids which develop into independent individuals it proceeds to give rise to segments that are reintegrated and brought under control, probably through the specialization of the nervous system. From this point of view the development of the annelid trunk appears to be a succession of incomplete reproduction processes, but which undergo reintegration into a whole consisting of segments.

In the light of all the facts it is difficult to avoid the conclusion that the origin of the dorsal lip region is fundamentally similar to these processes of physiological isolation, since the dorsal lip region arises secondarily at the posterior end of the embryonic area as a region of high susceptibility, increased activity, and rapid growth.

The secondary invaginations afford an interesting bit of evidence in that, with certain degrees of inhibition of the apical region, the range of dominance is decreased and after a first isolation has occurred—a second one may arise nearer the apical end.

3. *The Question of "Specificity" in Teratogeny.*—The word "specificity" as used in the literature on vertebrate teratogeny is difficult of exact definition. In general it has been used to designate some unique reaction of an organism or part, to a particular experimental factor, especially a particular chemical substance, such as to produce an anomaly characteristic of that individual external factor.

Various attempts have been made to identify experimentally produced abnormalities with either a specific chemical effect or with a more general physical effect of some one of numerous agents that have been used to modify development. The literature of this subject has been reviewed incidentally so many times (Korschelt and Heider, 1902; Hertwig, 1906; Jenkinson, 1909) that only certain essential facts and conclusions need be mentioned here. Gurwitsch (1896) attributed the various abnormalities of the frog produced by him in sodium bromide, lithium chloride, and weak solutions of strychnine, caffein, and nicotine, to an "einigermaassen specifische" action of individual chemicals upon certain regions of the egg. Stockard (1909) at one time believed that cyclopia in *Fundulus* was due to a specific cyclopic producing property of $MgCl_2$ and went so far as to suggest that cyclopia in man may be due to an unusually high amount of Mg in the amniotic fluid or in the blood of the pregnant mother. When, however, it had been shown that a large number of different substances produce the same anomaly, Stockard (1914) abandoned his earlier view that there are "specific responses to the given chemical substances employed" and suggested that since "a certain definite response on the part of the developing organism may be consistently obtained after carefully adjusted treatments with a large number of different substances . . . in certain cases they may serve simply to lower the developmental metabolism and thus prevent or arrest the formation of particular structures." No reason is offered to explain *how* lowering the developmental metabolism may cause the prevention or arrest of the formation of particular structures or *why*, under properly controlled conditions, *particular* regions of the embryo are affected more than other parts and in a definite and sequential relation to one another, though he notes that the nervous system and special sense organs are most affected by the use of chemical agents that inhibit development.

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[1] Child, 1911, *Jour. Exp. Zoöl.*, Vol. 11.

To digress for a moment, in the light of present knowledge we believe it possible to give a rational interpretation of these facts. The reason that a lowering of the developmental metabolism under conditions where acclimation does not occur, can prevent or arrest the formation of such structures as nervous system and special sense organs, is the fact that there is a gradient in the rate at which the processes described by the collective term "metabolism" are taking place along physiological axes. Anterior, medial, dorsal regions, because of their higher metabolic rate are more susceptible to the inhibiting conditions and hence are more affected.

Bataillon (1901) studied the abnormal development of the frog in isotonic solutions of cane sugar, NaCl, and a number of other salts and reached the conclusion that osmotic pressure and the consequent withdrawal of water from the developing embryo, is the effective cause of abnormalities. Jenkinson (1906) showed that solutions of a large number of substances, isotonic with 0.625 per cent. NaCl, do not produce the same, but markedly different effects. It may be pointed out however that these effects differ not in kind, but in degree, and are on the whole readily interpreted in terms of differential susceptibility. And it may be remarked here that the large number of abnormalities in the frog described during the past quarter of a century which have been produced by experimental means may all be rationally interpreted in these terms and readily brought into relation with the axial gradients. Jenkinson's conclusion was that the abnormalities produced—covering most of the modifications known in amphibian teratology—were not to be attributed to the osmotic pressure of the solutions but were due to their chemical or physical properties. Morgan who has done much work on abnormal development in the frog, stated, (1906) in speaking of the osmotic and chemical effect of salt solutions on the frog egg, that: "It is probable that the effect is a double one; in part chemical, in part osmotic."

There can be little doubt that certain substances especially in high concentrations, do have a physical as well as a chemical effect upon protoplasm. Furthermore, in a highly specialized egg like that of the frog where a considerable volume of relatively inert matter (yolk) is localized in one hemisphere, high osmotic pressures such as exist in solutions of certain electrolytes and other substances in concentrations high enough to cause markedly abnormal development, and substances that have a solvent action on the yolk (alcohol, *e.g.*), may in extreme cases set up secondary disturbances of a mechanical sort such as partly or wholly to obscure the general susceptibility relations. But, it is especially to be noted that those agents which, in extreme concentrations or intensities of action, do tend to obscure the differential susceptibility relations, may be used in other concentrations or intensities, to produce differential inhibitions that, in kind, are not different from the differential inhibitions produced in a variety of other agents.

Although it was stated (p. 346) that in the reaction of the frog egg to the different substances used to modify development, no evidence was found of any "specific" action, *in the sense in which the term has been used*, I do not wish to be understood as implying that all chemical substances act upon protoplasm in exactly the same way. It is to be expected, and bio-chemical and zoölogical literature contain abundant evidence to show, that different agents do affect protoplasm in different ways. KNC, *e.g.*, which is a powerful reducing agent is known to interfere with oxidations and enzyme activity in the organism. Hyman (1919) found that in planaria oxygen consumption may be reversibly cut down as much as 80 per cent. to 90 per cent., in the presence of minute amounts of KNC. In this paper she gives a comprehensive review of the literature on the action of cyanides on protoplasm so that further discussion of this matter is unnecessary here. So far as susceptibility relations are concerned formaldehyde appears to affect protoplasm in much the same way as KNC, and $KMnO_4$ which is a vigorous oxidizing agent is equally efficient for demonstrating differences in susceptibility in organisms.[1] It is a significant fact that these oxidizing and reducing agents are among the most effective agents that have been used in the study of physiological axiation.

As regards anesthetic action in the stricter sense, whether these agents disturb oxidations, affect the lipins of the cell, produce changes in the surface membranes, etc., there appears to be no consensus of opinion.

Various salts affect protoplasm, especially the colloid substances, in widely different ways. They may bring about changes in the state of aggregation of colloids, and the loss or addition of water; induce changes in ionization which may result in the precipitation or solution of certain substances in the protoplasm; or upset the electrical equilibrium, and so on.

Acids and alkalies also affect the speed and type of many reactions in protoplasm in a variety of ways depending upon the ionization constant of the acid or alkali, concentration, reactivity of the substances formed by their interaction with protoplasm, etc.

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[1] Child, Biol. Bull., 36, 1919.

It is obviously impossible here to go into a detailed discussion of the particular types of reaction set up between external agents and living protoplasm. For a resume of this important subject the reader is referred to Mathew's "Physiological Chemistry," especially chapter V., where references to the literature may be found.

In spite of the various factors concerned in the action of external agents upon living protoplasm, the highly significant fact remains that the susceptibility relations to external agents whose action is severe enough to kill within a few hours or to be distinctly toxic show not only a high degree of uniformity for at least a large number of different agents, but also a very definite correlation with metabolic conditions.

As regards permeability of limiting membranes there can be no doubt that it is an important factor in the movement of substances into and out of the cell. But the passage of many substances across the cell membrane in concentrations high enough to kill the protoplasm follows only upon the destruction of, or irreversible changes in the surface membrane and the consequent more or less complete disappearance of its special physiological characteristics. Furthermore, the cell membrane is alive and therefore metabolically active to some extent, and its peculiar properties as regards permeability are dependent upon the fact that it is alive, since they disappear with its death. It is evident then, first, that semi-permeability is more or less closely associated with metabolic conditions and second, that differential susceptibility to external agents cannot be interpreted in terms of permeability as distinct from metabolic conditions.

Experimental analysis has already shown that many of the so-called cases of specific action of external agents are not properly speaking specific effects at all, but actually are differences in degree rather than in kind. And the question may be raised whether at least many other apparently specific effects will not prove to be of the same sort.

Even admitting that different agents do attack the protoplasmic reaction system at different points—as they undoubtedly do—the important point is that in such a complex and intercorrelated physico-chemical reaction system as protoplasm, it is hardly possible to alter any one set of reactions or conditions to any considerable degree, without disturbing the system as a whole. Consequently in subjecting the frog egg or any other developing organism to an agent that in any way inhibits protoplasmic activity to a sufficient degree, it is certainly, in its broader aspects, not a specific, but a *general* or quantitative effect and reaction of the organism to the agent that becomes apparent. It is the striking uniformity of the susceptibility relations of organisms to so many different agents and conditions that forces the conclusion that there must be some fundamental feature of all axiate organisms that is affected in much the same way by different agents. In other words, *it is the existence of a gradient or gradients in metabolic rate, and protoplasmic conditions associated with them along physiological axes in the organism, that determine the uniformity and the susceptibility relations that have been shown to exist, and afford the basis for a rational interpretation of teratological development.*

VI. Summary.

1. In the ovarian egg of the frog there is a definite relation between the polar axis of the egg and its blood supply. In every case the pigmented hemisphere is more richly supplied with arterial blood than the unpigmented hemisphere. It is suggested that polarity arises in response to this external factor, viz., respiratory and nutritive relation of the egg to the parent body. It also seems probable that the localization of the pigment on the egg is in response to the greater oxygen supply over a restricted area. There is no orientation of the ovarian egg to gravity.

It is pointed out that polarity in a number of other organisms arises in response to conditions external to the egg.

2. It is shown that differences in susceptibility exist in different parts of the egg and embryo of the frog. These differences are evidenced in the following ways:

(*a*) By differential death gradients,

(*b*) By differential inhibition, and

(*c*) By differential acclimation and recovery.

3. The two modifications of the susceptibility method described in this paper, (*a*) and (*b*) above, agree in showing that:

(*a*) Those parts of the egg where development first begins and proceeds most rapidly die soonest in lethal concentrations or intensities of external agents and are inhibited most under conditions so severe that acclimation does not occur. Both disintegration processes and inhibition are differential, paralleling the polar axis in early development and other axes that arise later.

(*b*) All of the modifications, produced under different conditions, are essentially similar, differing not in kind but in degree. No evidence was found of "specificity," "blastolysis" or "disorganization" as effective factors in the production of any of the modifications.

(*c*) The experimental data indicate that any type of abnormality may be produced under the influence of any inhibiting agent by controlling the concentration or intensity of action, the length of exposure and the stage in development (physiological condition) of the egg or embryo when exposed.

4. Differential inhibitions, appearing under conditions that prevent acclimation, are evidenced in the following and other ways:

(*a*) By the relatively slower division of the more active animal pole cells with the result that the cell size ratio approaches and may become equal to one.

(*b*) By the retardation or prevention of downward movement of material from the animal hemisphere, which takes place, normally, most rapidly in the sagittal plane, but which under these conditions, is most retarded in this plane.

(*c*) By the retardation of the dorsal lip region with the formation of V-shaped blastopores and oval yolk plugs that may be several times longer in the sagittal plane than broad.

(*d*) By the retardation of both dorsal and lateral lips of the blastopore resulting in the formation of flat-crescent blastopores and a whole series of modifications between this and,

(*e*) Perfect equatorial gastrulæ.

(*f*) By the appearance of a secondary invagination apical to the original blastopore.

(*g*) By the shifting of the blastopore so that it may come to lie at or above the equator of the egg. (Accompanies *d*, *e*, *f*.)

(*h*) By the apparent obliteration of bilaterality, resulting in the formation of embryos radially symmetrical about the original polar axis.

(*i*) In the later embryonic stages a variety of abnormal types appear most of which are later stages of *c*, *d*, *e* and *g*, above. Among them may be mentioned, microcephalic forms with eyes, nasal pits, and ventral suckers in all degrees of approximation to complete "fusion"; anencephalic forms appear, and spina bifida of all degrees are common. Most of the forms show a marked dorsal concavity and the medullary folds may remain open in the brain region or throughout their entire length.

5. Since the dorsal lip region arises at the distal end of a rapidly growing region, and bears a definite spatial relation to the apical region that is experimentally controllable, it is probable that it arises by physiological isolation consequent upon the rapid growth in length, especially in the sagittal plane.

6. The data presented indicate that at the beginning of development, metabolic processes are most rapid at the apical (animal) pole and decrease toward the basal (vegetative) pole.

7. With advance in development a secondary region (dorsal lip) of high susceptibility appears, probably as a physiological isolation and represents a posterior "segmental" region of rapid growth similar in many respects to the posterior growing region of annelid larvæ.

8. It is pointed out that the existence of gradients in metabolic rate along physiological axes in the egg and embryo make possible the great uniformity and lack of specificity shown to exist in the susceptibility relations in the frog and other organisms, and affords the basis for a rational interpretation of teratogeny, not only in the frog but also in vertebrates generally.

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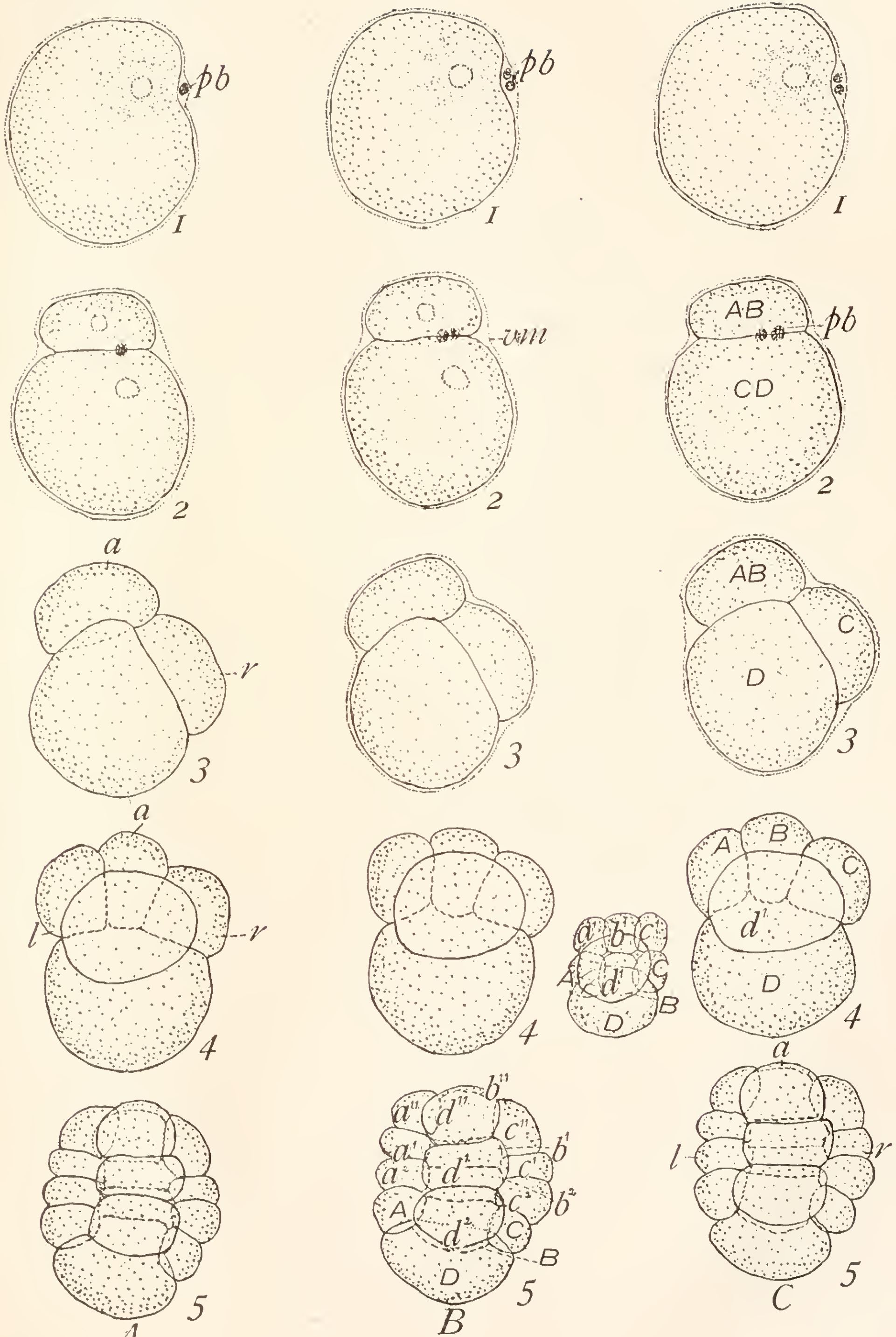
ERRATA.

Plates illustrating article by G. W. Tannreuther in the September issue. Should be inserted following page 208.

PLATE I.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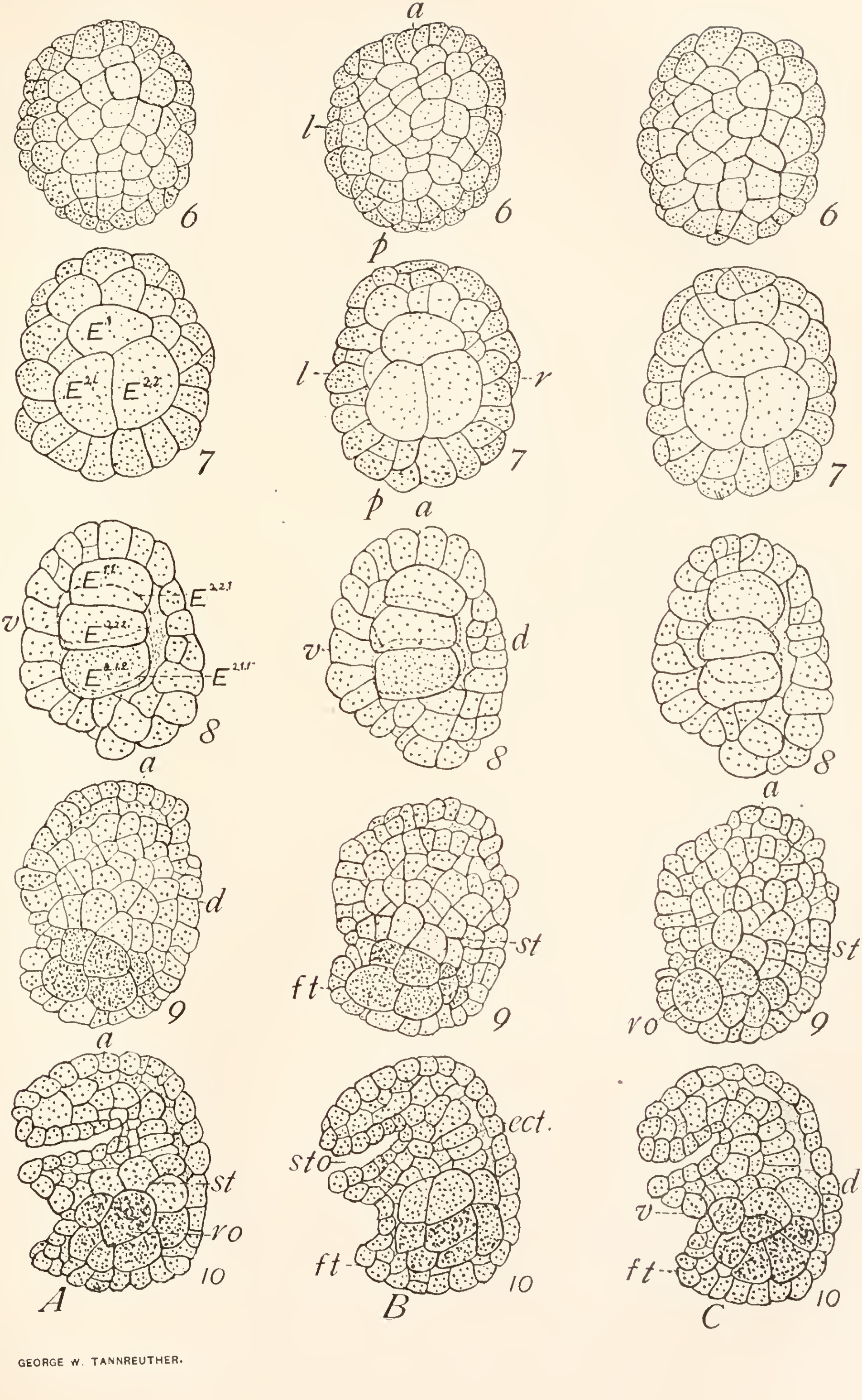
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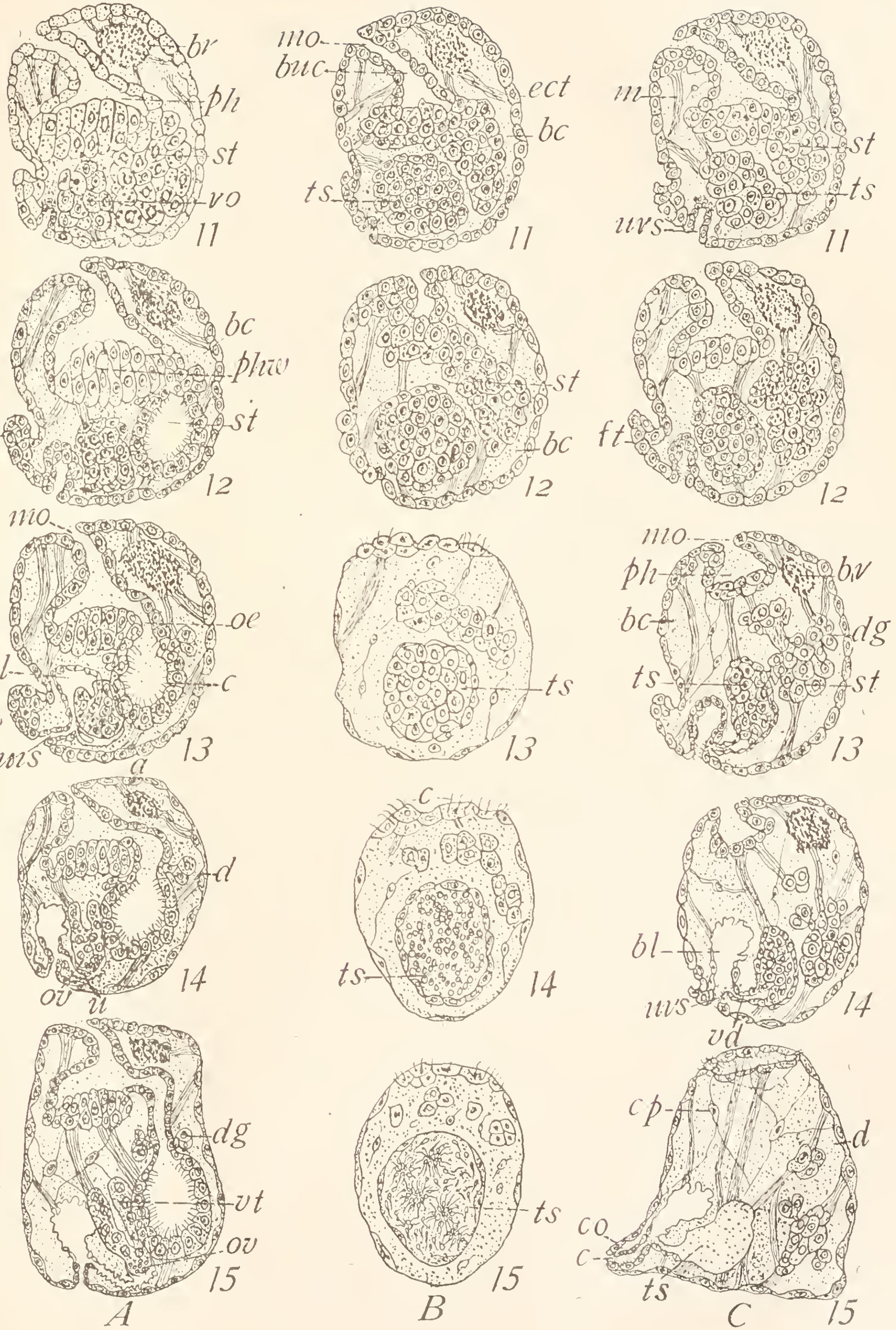
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BIOLOGICAL BULLETIN

THE ORANGE STRIPED ANEMONE (SAGARTIA LUCIÆ, VERRILL). AN ECOLOGICAL STUDY.

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The orange striped anemone (*Sagartia luciæ* Verrill) is one of the commonest of the marine invertebrates to be found along the Connecticut coast. It was first observed at New Haven in 1892 by Miss Lucy Verrill, and described in 1898 under the name of *Sagartia luciæ* by Professor Verrill, who believed that it had been introduced into this locality from more southerly waters of North America, possibly on the shells of oysters. From New Haven it has greatly extended its range, and in 1901 was reported as being found as far north as Salem, Mass.

The morphology and anatomy of *Sagartia luciæ*, as well as its general physiology and geographical dispersal, have been ably dealt with in earlier papers. The present contribution embodies the results of a summer's observations made at various points along the Connecticut shore of Long Island Sound, and is chiefly concerned with the ecological relationships of the creature.

Although *Sagartia luciæ* is one of the most numerous of the invertebrates of the littoral fauna of Long Island Sound, it is, nevertheless, an inconspicuous member of the fauna, both because of its diminutive size (being normally about a quarter of an inch, or less, in diameter) and because of the concealing character of its olive green coloration. The bright orange stripes, to the number of about twelve in adults, further aid in rendering the creature inconspicuous by interrupting the contour of the body. The tentacles are usually arranged in four rather ill-defined rows, each row consisting of twelve tentacles. Within each row there is contained one tentacle, larger and longer than the rest, which normally corresponds in location with an orange stripe. These larger tentacles, also, indicate the position, within the body, of the equally numerous intermesenterial spaces. Within the circle of tentacles lies the greenish oral disc, the center of which is pierced by the mouth opening, surrounded by the lip band. The latter structure is usually reddish in color; is impressed by a number of folds about its inner edge; and bears two prominent, opposite, gonidial grooves.

Since reproduction is often accomplished by longitudinal fission, or less commonly by budding, or by dividing at the base in a sort of budding process which Davenport (1) has termed basal fragmentation, individual anemones vary considerably in form and size, according to the stage of life which each is passing through at the time of the observation. Mature individuals which have not yet apparently begun reproductive activities, are illustrated by Figs. 1 and 2. Extremely unsymmetrical, and even monstrous, forms are frequently encountered, the results of accidental mutilation, for regeneration of lost parts reaches, with *Sagartia*, a high degree of development. Consequently surprisingly small fragments often settle down and regenerate an entire individual. Davenport has pointed out that new tissue, in individuals which are the results of reproduction by division, is of a hue of green less olive in tone that that of older individuals, and that the new stripes are a brighter yellow, with less orange tint. This is also true of new tissue which is being regenerated to make up for some lost portion of the body.

The general color scheme of *Sagartia* seems to be particularly well adapted to the concealment of the creature. The olive green of the body, broken by the longitudinal orange stripes, and the grayish white of the tentacles, forms a color aggregation of unusually subdued character when the animal is viewed in its normal habitat. When fully expanded, and hence in its most conspicuous attitude (Fig. 1), the body becomes lighter in color (because the more translucent) and the body assumes a translucency, as do also the tentacles. The latter, indeed, become, often, virtually colorless, and almost transparent. The color of the orange stripes likewise undergoes a diminution in intensity as the expansion of the animal progresses. Under these conditions, when viewed either from above or below (the latter by means of a mirror placed at the bottom of the pool), the body outline becomes almost impossible to trace. Another aid to concealment is afforded by the immobility of the tentacles when the animal is fully expanded, and also by the extreme deliberation with which the act of expansion takes place. When contracted (Fig. 2) the animal appears like a small dark greenish button-like protuberance, whose outline, however, is interrupted by the orange stripes. This combination of dark olive green and orange (which seems at first a rather gaudy *ensemble*) can be shown to be a peculiarly effectual one for the type of rock on which *Sagartia* is usually found seated, *i.e.*, the granites of the New England coast. The rock is flecked with black, and with varying tints and shades of gray, green, reddish, and yellow, from the included quartz, feldspars, hornelende, etc., and from the various chemical combinations with these minerals which the oxygen of the air and the substances of the sea water make.

Sagartia luciæ occurs below tide level on piles, or other submerged objects, and is frequently associated with the rock barnacle (*Balanus balanoides*), and with two species of mussels; the ribbed mussel (*Modiola plicatula*), and the black or edible mussel (*Mytilus edulis*). In the same company may also be found small clusters of the common oyster (*Ostrea virginica*). More often, however, the habitat of *Sagartia* are the tidal pools between the tide lines, among rocky headlands and shores, and rocky tidal islands, in which the New England coast abounds. Wherever it occurs, however, it will usually be found attached to vertical, or overhanging surfaces, rather than to horizontal ones (Fig. 3). In some few tidal pools it was found, however, on the gently sloping sides. Comparatively few enemies, either organic or inorganic, attack those individuals which are attached to the vertical or overhanging sides of the pool, while those that becomes detached and cast to the bottom are soon either buried by debris brought in by wave or tidal currents, or fall an easy prey to mud crabs (*Panopeus*), rock crabs (*Cancer irroratus*), or hermit crabs (*Eupagurus longicarpus*), for *Sagartia*, despite its armament of nettling acontia, is without means of defense against these heavily armored scavengers.

As a rule, the rock itself seems to be the favorite location of

Sagartia, for the creature can detach itself, at will, from its seat, and move by means of its tentacles, or float about, head downward, from the surface film of the water until a more favorable locality is found. The fact that the rock, or other seat of equal solidarity, is chosen more often than any other for the obsession of *Sagartia* would seem to indicate that the creature possesses a sense which instructs it regarding the relative stability of submerged objects. However it is often found attached to the shells of living mussels and even sometimes upon eel grass, fucus, or other algæ. It often occurs, also, attached to shells, and other objects that have become firmly wedged into crevices in the rock by the action of the waves.

Sagartia is ordinarily solitary in its habit, and not communal, and yet, what seem to be in effect, colonies, at least in so far as mere physical propinquity is concerned, were met with in tidal pools, where, apparently by repeated divisions, coupled with but little migration on the part of the increasingly numerous progeny, groups of from ten to twenty-five individuals, with their bases almost in contact, were formed. That this group formation aided in securing food was seen when large beach fleas (*Orchestia agilis*), which were easily able to escape the embrace of a single anemone, were at once, caught and held by the numerous tentacles brought to bear in the capture by several contiguous individuals at once, and easily nettled into a state of helplessness. Possibly such a colonial existence (if it may be so called) makes also for the more effectual protection of the individual members of such a community. Such groupings seemed to be the exceptions, and not the rule, in the disposition of the anemones in the tidal pools. It is interesting to conjecture, that possibly this grouping, perchance accidental at first, marks the beginning of the development of a movement toward communal living on the part of *Sagartia*. If this manner of living does make for the better protection of the members of colony, and aids in securing more frequent and larger captures of food, then we may suppose that the collective individual will thrive at the expense of the independent one, a law of progress derivable from other fields also than the biologic one.

The food of *Sagartia* consists mainly of small crustacea, annelids, etc., which are seized by the tentacles, rendered innocuous by the acontia and then gradually manipulated into the mouth. Fig. 4 represents an individual capturing a beach flea, which had been steered into the blossom of tentacles with a broom splinter. All sorts of sufficiently soft food substances, either living or dead, are taken undiscriminatingly. Numerous individuals were fed by the writer, both in their natural habitats, undisturbed, and in aquaria, with pieces of fish, both fresh and decaying; clam, mussel, beef, bread, salt pork, insects, etc., which the anemones seized and devoured with the same impartial gusto which they showed for living creatures. They however rejected hard substances, such as sand grains, bits of shell, wood, etc. Beef juice, dexterously squirted upon one portion of the blossom of tentacles with a finely drawn out pipette, caused them to respond at once by throwing over a large number of tentacles to that side whence the stimulus had come and endeavoring to entrap some object. Frequently the tip of the pipette would be grasped, only to be relinquished again. Foreign substances, *i.e.*, those not usable as food, were pushed to one side of the blossom of tentacles and allowed to fall off. The food which seems to form the bulk of their intake consists of the smaller crustaces, such as small beach fleas (*Orchestia agilis*), small clam worms (*Nereis*), and other small marine worms, very small crabs in the soft shelled stage just after a molt, chiefly the mud crab (*Panopeus*), crushed rock barnacles (*Balanus balanoides*), very small fish fry, and heterogeneous particles of animal tissue which the tidal and wave currents chance to float by. A lessening of the food supply in some particular neighborhood apparently is the moving cause that sets many individuals roving by hanging head downwards from the surface film of the water and being borne here and there by currents until a more fruitful locality is discovered. Rarely there was found a more ambitious individual which had fastened upon the shell of a periwinkle (*Littorina littoria*), and was continually being carried into pastures new.

The enemies of *Sagartia* are numerous. Among them the most important are: various species of rock bottom feeding fishes, rock and mud crabs, starfishes (*Asterias*, and others), and the larger clam worms. Many individuals are doubtless swept away by the assaults of sand and debris-laden waves in times of storm, and by the grating of ice cakes against the rocks during the winter. Frequently a tidal pool is drained by an excessively low ebb tide, and the anemones left exposed for an hour or so to the rays of a direct sun. This, coupled with a vigorous dry wind will often accomplish the destruction of some individuals, though they were able to withstand even such adverse conditions to a surprising degree, as experiments showed.

It seems remarkable that so soft and relatively defenseless a creature should have so greatly increased its numbers and extended its range in our waters. Its success in meeting the unusually numerous vicissitudes of a littoral existence may perhaps be attributed to the following: (1) in ability to withstand considerable differences in temperature, (2) its ability to withstand buffeting by the waves because of the yielding and resilient character of its body, (3) its ability to contract tightly, and to survive through a period of foul water, or of dry conditions exposed to the sun and wind, (4) its apparent disregard of differences in the salinity of the water, (5) its protective coloration, (6) its defensive acontia, (7) its rapid rate of reproduction and growth to maturity, (8) its several methods of reproduction, (9) its ability to withstand annihilation through laceration, and (10) its ability to regenerate lost parts.

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EXPLANATION OF PLATE I.

FIG. 1. A fully expanded, mature *Sagartia* with its tentacles in exploring posture, after having been stimulated with beef juice injected into the water. The object to which it is attached is an edible or black mussel (*Mytilus edulis*).

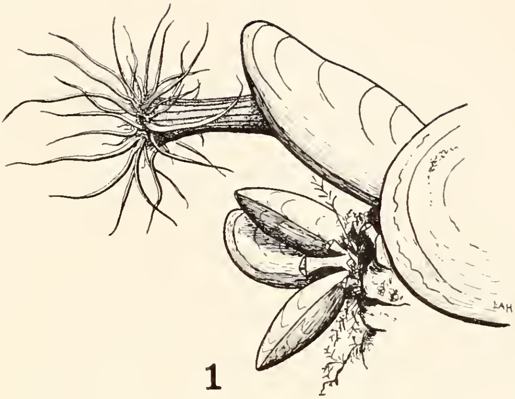
FIG. 2. Contracted mature *Sagartia*.

FIG. 3. Transection through a typical ideal tidal pool, to show the disposition of *Sagartia* individuals, and their general ecological relationships.

1. *Sagartia luciæ*.
2. Edible or Black Mussel (*Mytilus edulis*).
3. Ribbed Mussel (*Modiola plicatula*).
4. Mud Flat or Elephant Snail (*Nassa obsoleta*).
5. Oyster Drill (*Urosalpinx cinerea*).
6. Periwinkle (*Littorina littoria*), not numbered in the figure. It occurs in great numbers, everywhere.
7. Oyster (*Ostrea virginica*) rare in tidal pools.
8. Clam Worms (*Nereis*), in mud among masses of mussels.
9. Small Ribbon Worms (*Mechelia*).
10. Starfish (*Asterias forbesi* and *vulgaris*).
11. Hermit Crabs (*Eupagurus longicarpus*).
12. Mud Crabs (*Panopeus herbestii* and others).
13. Rock Barnacle (*Balanus balanoides*).
14. Green Beach Flea (*Orchestia agilis*).

FIG. 4. *Sagartia*, capturing a small Beach Flea (*Orchestia agilis*).

FIG. 5. Partially contracted *Sargtia*, of large size, just before fission. A strong light had been placed behind the creature, revealing the manner of the accommodation of the contracted tentacle blossom, and the folded sides of the gastrovascular cavity. The latter is seen to be empty of food.



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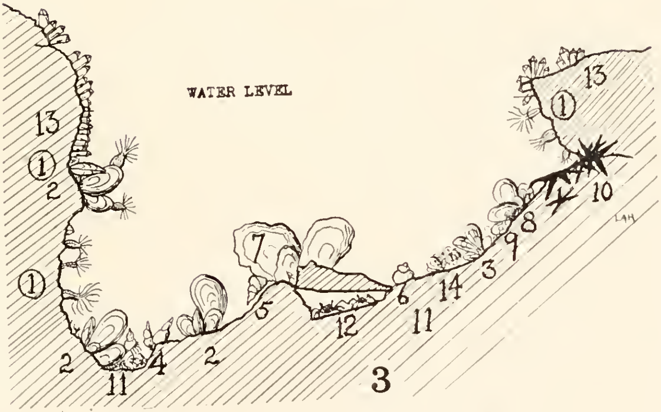
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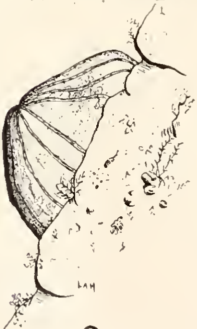

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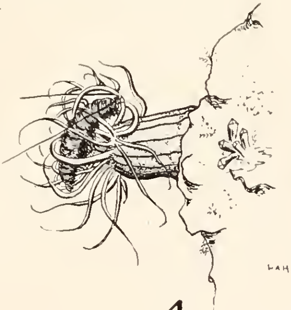

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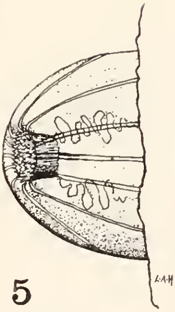

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LEON AUGUSTUS HAUSMAN.

PRELIMINARY NOTE ON THE SPERMATOGENESIS OF PEDICULUS VESTIMENTI.[1]

KATHARINE FOOT.

In response to a request from Col. Alexander Lambert I came to Paris, December, 1917, in order to study *Pediculus vestimenti* from the point of view of the biologist in the hope of getting some data that might be of service to the investigators who were studying the louse as a possible transmitter of certain diseases prevalent among the troops.

In an exhaustive and masterly study of the problem in relation to trench fever, Col. Strong has shown that the lice can unquestionably transmit this disease and has proved beyond question what was heretofore merely a surmise.

Such data as I have been able to collect that may have some bearing on the problems in relation to disease have been reported to the Research Department of the American Red Cross; but I have omitted from such reports purely cytological data which have no apparent practical value.

As far as I know, no report has been published of any work done on the chromosomes of the louse, and as *P. vestimenti* has been classed as an Hemipterous insect I was interested to see if the chromosomes have the same bizarre morphological characteristics as are typical of so many Hemiptera.

The spermatocyte chromosomes are very minute, so minute that I have as yet found it impossible to demonstrate the method of division of the second spermatocytes; but I have several entirely satisfactory stages of the first spermatocyte chromosomes, and as these have the same morphological characteristics as the corresponding stages in other species of Hemiptera, it is quite permissible to assume a like correspondence for the second division.

[1] My grateful acknowledgments are due to the late Professor Blanchard, who gave me a most cordial welcome to his laboratory at the Ecole de Médecine. He not only gave me ample space for my work but the sympathy and encouragement I received from him and the members of his staff made it possible for me to continue my investigations of these repulsive insects.

The above mentioned morphological peculiarity of the chromosomes of many Hemiptera *i. e.* the unequal bivalent—called by Wilson the X Y chromosome—is present in the first spermatocytes of *Pediculus vestimenti* and its division is typical—the two unequal parts dividing as univalents in the first division. The unequal bivalent is demonstrated in the metaphase stages of Figs. 10, 11, 12, and the division of the smaller half of the unequal bivalent is shown in Fig. 13.

Miss Strobell and I (1914) published a series of photographs showing both the first and second spermatocyte divisions in *Euschistus variolarius*, *Euschistus servus* and in two generations of hybrids from *E. variolarius* by *E. servus*. A comparison of these photographs of the first spermatocyte chromosomes with the above mentioned Figs. 10–13 will show that the first spermatocyte chromosomes of *Pediculus vestimenti* are of the same type as in the species of *Euschistus* referred to, and it is therefore quite logical to assume that the second spermatocyte chromosomes are equally typical though its demonstration is not yet possible.

In Fig. 16 (on the right) is a group of chromosomes from an embryo in an egg at the basal end of the ovary.

It is not possible to determine the exact number of chromosomes of this group, as 18, 19 or 20 can be counted.

The first spermatocyte chromosomes indicate that the somatic number should be ten and in the few spermatogonial groups I have found it possible to identify ten chromosomes; but they are so small and so frequently constricted that the estimate can always be questioned.

I am convinced that the most favorable stage for an exact interpretation of the louse chromosomes is the first oöcyte prophase, but to secure this stage involves a patient search which cannot be undertaken at present. Fig. 18 indicates that it will be possible to find the later prophase stages, for in this preparation the chromosomes are nearly formed, and it ought to be possible to find the slightly later stages in the same locality of the ovary.

An early stage of the yolk spheres of the ovary is shown in the photomicrograph of Fig. 16 (on the left). These are chromosome-like structures, which in the early stages select the chromatin stains and are morphologically so like chromosomes that one is tempted to interpret them as chromosomes which develop into yolk-spheres.

A series of stages prior to the first spermatocyte metaphase is shown in Figs. 1 to 9. The centrosomes of the first spermatocytes (Figs. 1 to 4) are a feature of these stages which is most marked. The position of these centrosomes in relation to the nucleus is very variable: they are in contact with the nucleus or almost in contact with the periphery of the cell or in any position between these two extremes. In later stages they are frequently divided into two or more, rarely into three, parts. Several of the former are shown in Fig. 7 (the centrosomes of this figure are a little exaggerated by the artist).

The Development of the Spermatid into the Mature Spermatozoön.—The most striking feature in the development of the spermatozoön is the duplex character of the tail. Apparently the tail is composed of two distinct and independent filaments, the apparent independence of these filaments being more marked in the earlier stage (Figs. 20–21). One would naturally suppose that one of these filaments represents the flagellum of other forms, but it has not been possible to demonstrate any substance connecting the two filaments, though the fact that even in smear preparations the two are never found widely separated would indicate that they are attached by some connecting substance.

HISTORICAL SKETCH.

Certainly no insect has been accused of being the promoter of a greater variety of diseases than the louse and perhaps no accused has been charged with so many crimes on less evidence. *Pediculus* has been credited with transmitting the following diseases: typhus fever, typhoid fever, recurrent fever, trench fever, tuberculosis, spinal meningitis, plague, leprosy, beri-beri and more than a dozen minor skin diseases. In some of these cases the evidence seems to be confined to the fact that the patient may be infested with lice.

For typhus fever, recurrent fever and trench fever it has been proved that the lice do in fact transmit these diseases, but details as to the method of transmission are still disputed. It is held by many investigators that germs are not transmitted by the bite of the louse—the sole method of transmission being infection from their excrements. These are freely deposited on the skin and in the clothing of the host and subsequent scratching of the skin induced by the intense itching of the bites not only lacerates the surface but frequently causes a deep wound that leaves a scar lasting many months. It is self-evident that such lacerations over surfaces more or less infested with the excrements of the lice may cause a most effective inoculation. This method of infection by the fæces or the crushed body of the louse has been demonstrated for typhus fever by Nicolle (1909), Nicolle, Conte and Conseil (1910) and others. For recurrent fever by Sergent and Foley (1910), Sergent, Gillot and Foley (1911), Nicolle, Blaizot and Conseil (1912) and others. For trench fever, by R. P. Strong (1918). Familiarity with the feeding habits of the louse demonstrates the danger of this method of inoculation, for the amount of excrement discharged by each louse is surprising. I have frequently seen a single louse, during one hour's feeding, discharge excrement ten times, and five times is not unusual. For more than a year I have closely studied the feeding habits of *Pediculus vestimenti* and in my report to the research department of the Red Cross I described their behavior as follows: "Observations made during the feeding hour demonstrate that individual lice may behave very differently. As a rule they bite at once when young and vigorous. Some become gorged with blood in ten minutes and will not bite again, though most frequently they bite several times during the hour, moving around rather restlessly between times. Others bite continuously the entire hour, casting their excrements while biting. The old lice frequently do not bite for several minutes or even half an hour and then suck the blood very deliberately." I am inclined to believe that the method of biting demonstrated for one hour indicates the method for the entire twenty-four hours and that therefore the younger lice are almost continuously feeding on the host, wandering about and biting very frequently.[1] This would accord with observations made by Miss Strobell and myself on other species of Hemiptera, *Euschistus variolarius*, *E. servus*, *E. ictericus*, etc., etc. These insects were fed on fruit and could be closely observed during the twenty-four hours. They fed almost continuously during the night as well as during the day, and this leads me to surmise that the lice may feed with equal frequency and explains the torment that soldiers suffer even when infested with relatively few lice and suggests the possibility of inoculation from a single louse.

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[1] These observations support Nuttall's (1917) conclusions as to the probable feeding habits of lice. He thinks they bite very frequently, for when raising them on his wrist he noticed they started to bite at all times when he was quiet.

The disputed question whether simply the bite of the louse can inoculate the host has given rise to much discussion, some investigators emphatically denying that any danger is caused by the bite alone. Colonel Strong (1918) conducted some experiments with the aim to determine this point and concludes that "it seems fair to argue that the bite is probably a common mode of infection." He states that in some instances the disease was produced by pure biting experiments. His summary of the probable methods of infection through biting is as follows:

1. By piercing or stabbing and inoculating with mouth-parts contaminated with infected material such as blood from the patient or by louse fæces and body juices.

2. By stabbing and inoculating from the skin which has been contaminated with infected material such as louse fæces, and possibly body juices.

3. By stabbing and inoculating with mouth-parts which have been contaminated with virus grown or developed in the stabber-sac.

4. By stabbing and regurgitating of the virus from the alimentary canal.

5. By stabbing and the injection of the virus contained in the salivary juices.

6. By hereditary infection.

One of the difficulties in determining the value of the bite alone is to eliminate the fæces from the experiment. Those cases in which this is assumed to be done by allowing the louse to bite through chiffon do not appear to me to be conclusive, for in my experience they will not bite unless the chiffon is pressed upon the surface with sufficient strength to force the skin through the interstices of the chiffon, in which case the only effect of the chiffon is to reduce the area of the skin exposed, and the lice wander over the exposed area distributing the fæces as usual.

The danger of the louse as a promoter of disease has been so long appreciated that he has claimed the attention of a large number of investigators, the French and English forming the majority. The work accomplished up to 1917 has been most ably presented by Professor Nuttall, of Cambridge. His bibliographical list is an index of the thoroughness of his historical study of the subject. He has listed nearly 600 investigators.

A second historical sketch was published in France the same year (Souéges et du Noyer, 1917). These two studies are a convenient record of all the historical data that can be of value to the investigator.

Two English investigators (Warburton, 1909, and Fantham, 1912) were the first to study the life history of *Pediculus* and their results were supported and extended by Bacot in 1916. He determined the number of moults to be three, the average length of life of the louse, the average number of eggs deposited daily by a single female and other details, all of which my investigations support although our methods of work differed materially. He used an entomological box containing a number of lice and strapped this box on his person each night, allowing the lice to bite from six to seven hours daily. My lice were fed only one hour in the twenty-four and in such a manner that I could watch them while feeding.

The most serious difficulty in the investigation of lice is the food supply. In all the accounts with which I am familiar the investigator has had sufficient self-abnegation to feed his lice on his own person, but not having reached those heights myself, my initial difficulty was to find a host. There seems to be something extremely ridiculous in the mere suggestion of feeding a louse, for my most serious and generous offers received the discouraging response of a broad grin and an emphatic shake of the head. I finally succeeded in securing a host at the Asile de Nuit—a night employee of that institution. He was an old sailor whose evident familiarity with *Pediculus* at the Asile de Nuit had led him to cease to regard them in a humorous light, and he proved to be a thoroughly dependable food supply. He never missed his daily hour in my laboratory for the five months I employed him.

When feeding the lice I at first used the usual method of putting a number in a tube, inverting the tube on the arm and holding it securely in place to prevent the lice from escaping. I found this method unsatisfactory for several reasons and devised therefore quite a different technique. Lice cannot crawl up a glass surface if it is clean and are therefore perfectly safe in a glass ring even if it is only 2 cm. high. I had such rings made to order and fastened them securely onto the arm with melted paraffine. In this manner several different experiments can be conducted at the same time and the generations can be kept separate—further the lice can be conveniently studied with a lens during the hour they are feeding. For the remaining twenty-three hours they were kept in a Pasteur incubator at a temperature between 27° and 29° C. While in the incubator the lice were kept in cages such as those used in the laboratory for raising various insects. This cage is the tube de Borel, in which is placed an inner tube for the insects, this being held in the center by absorbent cotton which is kept wet to insure sufficient moisture. I found the use of absorbent cotton very inconvenient and replaced it with a short tube having an aperture large enough to contain the inner tube and open at both ends with a lip at each end sufficiently wide to center it in the tube de Borel. The inner tube in which the insects are kept is dropped into this shorter tube and an inch of water kept in the tube de Borel. I found this method a great economy of time, for it was necessary to pack the cotton around the inner tube with much care, since if the opening came in contact with the inner surface of the tube de Borel (often quite wet) a drop or two sometimes dripped into the inner tube and cost the life of one or more nymphs.

Several years of experience in crossing and raising other species of Hemiptera have been my guide in raising the lice. Miss Strobell and I found that the species we studied required as much humidity as possible while avoiding any condensation of the moisture. This I have found true for lice—a half a drop of water or less can kill a nymph. If he gets on his back on the glass in even a fraction of a drop of water, he cannot regain his feet until the water dries and if the glass is not clean he adheres to it and finally dies.

After trying the usual method of keeping the lice on small pieces of woollen or muslin cloth it occurred to me that a large number of short pieces (about 8 mm.) of soft, coarse thread would have many advantages. First they would be much more sanitary for they can be changed every day if necessary without disturbing the lice at all. When lice are on a small piece of cloth, the cloth becomes filthy in a few days and it is exceedingly difficult to remove the lice to fresh pieces. Further the thread avoids all the difficulties encountered in transferring and counting the lice. They cling to a thread with great tenacity; therefore single lice can be carried on a thread any distance with perfect safety. They deposit their eggs on the thread and therefore the eggs deposited each day can be conveniently collected and isolated. Using these threads made it a simple matter to record the following life history of a single pair of lice. The pair was hatched from eggs deposited in the laboratory and had their third (final) moult August 19. They were seen mating August 22. The next day 4 eggs were deposited and thereafter 4, 5 or 6 were deposited daily until the female died.

Record Fl. D.

1918.		Eggs.	Hatched.
Aug.	(Mated Aug. 22)		
23	Mated	4	
24		3	
25		5	
26		4	
27		4	
28		5	
29		5	
30		4	
31		5	
Sept.			
1		5	
2	Hatched the 10th day	6	1
3		5	3
4		5	4
5	(Mated)	5	3
6	(Male dead—each daily deposition of eggs kept separate from death of male)	5	7
7		5	3

1918.		Eggs.	Hatched.
Sept.	(Mated Aug. 22)		
8		6	5
9		5	5
10		5	5
11		6	4
12		5	5
13		5	6
14		5	4
15	Female dead	1	5
		113	
16			5
17	5 hatched from eggs deposited 9–7		6
	1 " " " " 9–8		
18	3 hatched from eggs deposited 9–8		4
	1 " " " " 9–9		
19	2 hatched from eggs deposited 9–8		6
	3 " " " " 9–9		
	1 " " " " 9–10		
20	1 hatched (the last) of eggs deposited 9–9		7
	3 " from eggs deposited 9–10		
	1 " " " " 9–11		
	2 " " " " 9–12		
21	1 hatched (the last) of eggs deposited 9–10		11
	5 " (the last) " " " 9–11		
	2 " from eggs deposited 9–12		
	3 " " " " 9–13		
22	2 hatched (the last) of eggs deposited 9–13		5
	2 " from eggs deposited 9–14		
	1 " " " " 9–15		
23	3 hatched (the last) of eggs deposited 9–14		3
	The last egg deposited 9–12 not hatched, nymph partly emerged but dead.		

Summary.

If we omit the one egg deposited the day the female died the daily average for the remaining 112 eggs is 4.9.[1]

113 eggs were deposited from August 23 to September 15 and all of these developed to the nymph stage, though six failed to completely emerge from the egg.

The above record shows that the male died September 6 and the female died September 15. From September 6 each daily deposition of eggs was isolated to determine how long the eggs were fertilized after the last mating (September 5). The record shows that all the eggs developed and that all hatched but one.

---

[1] Bacot found the daily average to be 5.1 and Nuttall's experiments demonstrated that under natural conditions, *i.e.*, when the lice lived continuously on the host, the daily average increased to 9.7.

As the female had her third moult between the 18th and 19th of August, she lived only 28 days after maturity, less than the average length of life for a female; but other females in the laboratory kept under the same conditions lived 42 days, 40 days, 39 days, etc. Bacot found the average length of life of a female to be 34 days. If my lice are a little below this average I think it is probably due to the difference in feeding—he fed his lice six or seven hours daily and mine were fed only one hour daily.

According to my experience, the longer a race is bred in the laboratory the less prolific they become and the death rate is much higher. I believe this is due entirely to an abnormal lack of nourishment. Feeding only one hour in twenty-four is certainly very abnormal for these insects.

One cannot study the record of the large amount of experimental work done on the louse without being impressed with the need of feeding these insects apart from the human host before certain problems now in dispute can be solved. My efforts have been largely given to this well high hopeless task which is my apology for a very superficial study of the spermatogenesis.

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EXPLANATION OF PLATES.

All the sketches were drawn at a magnification of about 750. Zeiss hom. immer. 2 mm. 140—apo. oc. IV camera lucida.

TECHNIQUE.

Smear preparations stained with May-Grünwald followed by Hollande.

These stains were used in accordance with the method published by Dr. Langeron (1916) and I am greatly indebted to him for many valuable suggestions and encouraging interest in my work.

PLATE I.

FIGS. 1, 2, 3, 4. First spermatocyte rest stages, each showing a distinct centrosome in varying proximity to the nucleus. No cell membranes are differentiated.

FIG. 5. First spermatocyte nucleus showing a single nucleolus and the chromatin segregating to form the chromosomes.

FIG. 6. Two first spermatocyte nuclei with granular chromatin segregating to form the chromosomes. The nucleolus has disappeared.

FIG. 7. Numerous first spermatocyte nuclei showing successive stages of the differentiation of the chromatin. In the earlier stages the chromatin is apparently homogeneous and later it is granular and segregating into definite masses to form the chromosomes. A few centrosomes are differentiated.

FIG. 8. Numerous first spermatocyte nuclei showing later stages than those of Fig. 7. In many of the nuclei the chromatin has segregated into 5 distinct masses foreshadowing the 5 bivalent chromosomes of the first spermatocyte metaphase.

FIG. 9. First spermatocyte nuclei about the same stage of development as those of Fig. 7.

FIGS. 10, 11, 12 and 13. Each figure shows the 5 bivalent chromosomes of the first metaphase. In each figure one or more of the chromosomes is a dyad, foreshadowing the first division. An unequal bivalent, which is typical of so many Hemiptera, is clearly shown in each group.

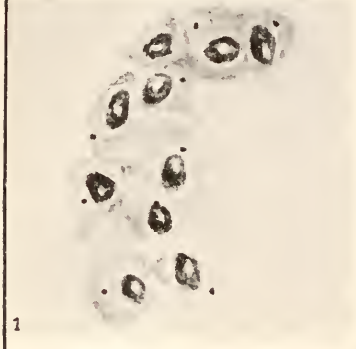
FIG. 13. All the chromosomes of this first metaphase group are dyads, foreshadowing the division of each. The large and small chromosomes of the unequal bivalent are detached and each is a dyad. This indicates that each will divide in the first division and that therefore the resulting halves will undoubtedly separate in the second division in the manner typical of so many Hemiptera.

FIG. 14. Late anaphase of the second division. The chromosomes are too small and too closely segregated to determine their number and form.

FIG. 15. Three telophases of the second division. Each shows an unequal division of the chromatin, this being the sole evidence, at this stage, of the separation of the large and small moieties of the unequal bivalent.

FIG. 16. On the left a photomicrograph of a small group of chromosome-like structures from an immature ovary. From these the yolk-spheres are developed. × 450. On the right a sketch of a group of chromosomes from an embryo in an egg at the basal end of the ovary.

FIGS. 17, 18. Two germinal vesicles from young ovarian eggs. In Fig. 17 the chromatin has partly segregated into threads, and a single nucleolus is present. Fig. 18 shows numerous small dense nucleoli, and the separate chromatin threads suggest a progressive step in the forming of the chromosomes though their abnormal number may be due in part to the technique.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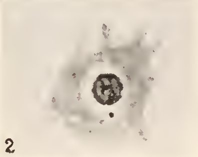
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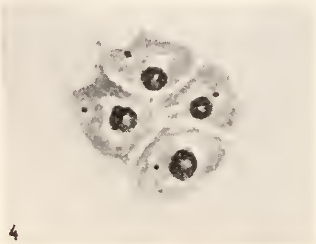
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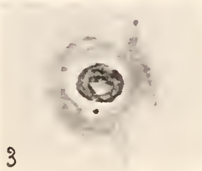
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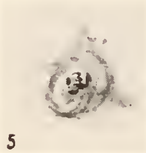
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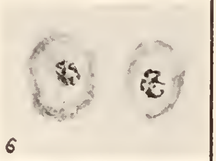
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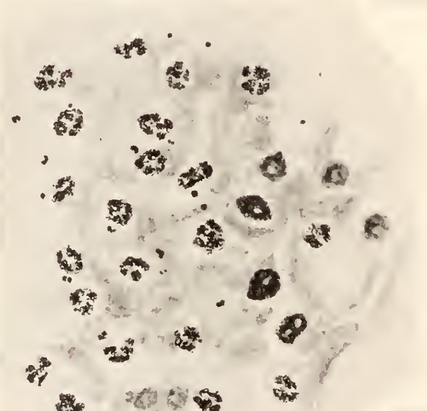
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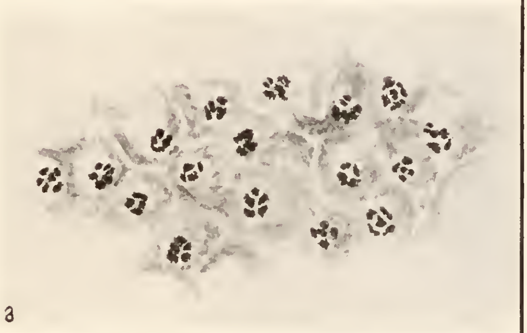
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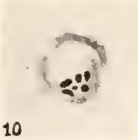
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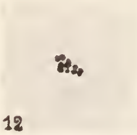
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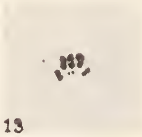
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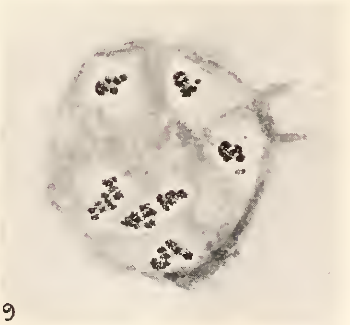
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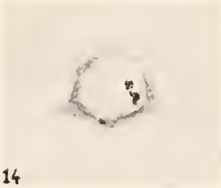
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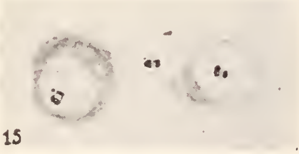
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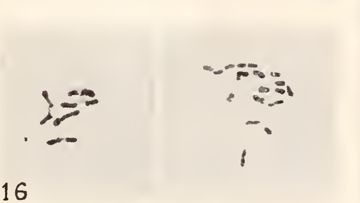
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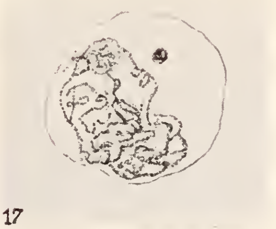
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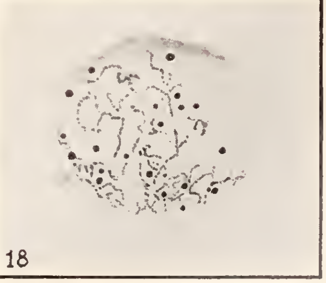
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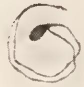
KATHARINE FOOT.

EXPLANATION OF PLATE II.

FIG. 19. Numerous heads of spermatids shortly after the second division. They are at first round, as demonstrated in the figure, and, as a rule, elongate before the appearance of the tail. In the elongated heads of this figure the middlepiece (?) is in evidence and from this point the tail develops.

FIGS. 20 to 27. Successive stages of the development of the spermatid into the mature spermatozoön. In Figs. 20 and 21 the middlepiece is demonstrated, the tail developing from this point. In Figs. 22 and 24 both the middlepiece and the acrosome (?) are demonstrated, the spine developing from the latter. Fig. 25 shows an early stage of the development of the spine. Figs. 26 and 27 demonstrate the mature spermatozoön in which the head and spine are fully developed. At this stage the middlepiece and acrosome are obscure.

19.



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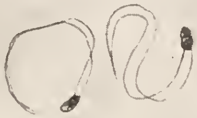

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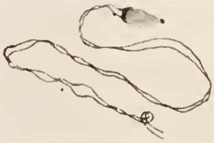

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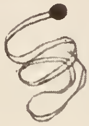

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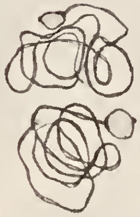

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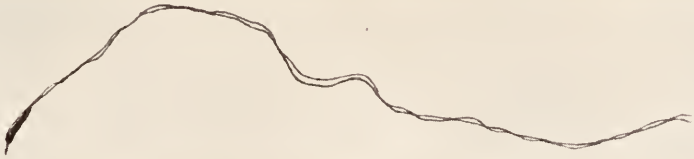

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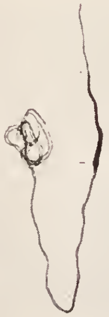

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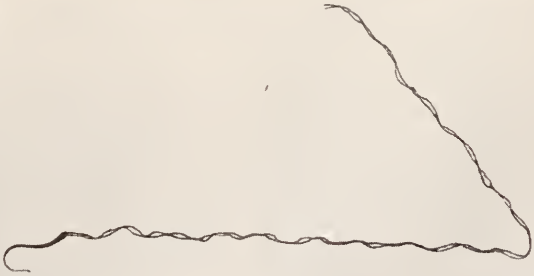

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KATHARINE FOOT.

DETERMINATION OF THE SEX OF THE OFFSPRING FROM A SINGLE PAIR OF PEDICULUS VESTIMENTI.

KATHARINE FOOT.

Nuttall (1917) gives a summary of the data as to the proportion of the sexes in *Pediculus vestimenti*. He says "the proportion of the sexes as determined by raising experiments has yielded contradictory results," and this he thinks is due to the small number of the broods of the experiments. In his own experiments of mixed pairs he found the sexes nearly equal, though his broods also were small.

Hindle (1917) discovered a marked inequality in the proportion of the sexes of certain pairs, and he concludes this to be the normal condition, basing this conclusion on the determination of the sex of 25 single pairs.

It has been proved by the observations of several investigators that a single female may deposit from one hundred to nearly two hundred eggs. It is obvious, therefore, that the sex must be determined for at least one hundred of a generation or the results are inconclusive. Certainly at least 75 per cent. of the eggs deposited must hatch and the sex be determined for these nymphs.

Hindle secured such a small number of individuals from each of his 25 pairs that Nuttall seems more than justified in questioning his conclusions. The number of individuals in each of the 25 broods studied by Hindle is as follows: 2, 4, 6, 9, 8, 10, 11, 12, 15, 17, 24, 25, 26, 26, 27, 29, 30, 31, 32, 34, 36, 38, 45, 48, 64. Fifteen of these 25 broods were all males or all females, *e.g.*, the brood of two were both females, the brood of four were also females, one of the broods of nine were males and the other brood of nine were both males and females. The brood of sixty-four were all females but as they probably do not represent more than 50 per cent. of the offspring from that pair, the evidence, even in this case, is without force.

Methods and Results.

In order to determine the sex of as large a number of the offspring as possible I adopted a different method from that of other investigators. Instead of waiting for the nymphs to mature in order to determine the sex, they were dissected at any stage that was convenient and those that died were not discarded but dissected at once and the sex recorded. It was thus possible to determine the sex of nearly all that were hatched.

Our method of treating these insects makes it possible to collect and isolate the eggs deposited each day, thus keeping an accurate count of the number of eggs deposited daily and the number hatched.

Both the male and the female had the third moult in the laboratory, the female July 22, 1919, and the male July 24.

The male died August 26; he lived therefore 33 days after maturity, thus exceeding the average length of life of *P. vestimenti* males. The female died September 5. She lived therefore 45 days after maturity, eleven days longer than the average length of life of the females. She continued to deposit eggs daily after the death of the male. She deposited in all 143 eggs (116 before the death of the male and 27 after that date). She commenced to lay eggs the 4th day after maturity and stopped laying 2 days before she died. She deposited 2, 3, 4 or 5 eggs each day. Of the 116 eggs laid before the male died, 11 failed to hatch, but nearly all developed and three of the nymphs partly emerged. Of the 27 eggs laid after the male died, seven failed to hatch, but all were fertilized and four of the nymphs partly emerged.

The nymphs were raised in lots of 20, and the death rate was relatively low in lots 1, 2, 3 and 4. The death rate in lots 5 and 6 was much higher. Several of the nymphs died even before the first moult. In some of these cases the nymphs were too dry to dissect, and therefore the sex was undetermined. This higher death rate was probably due to the age of the female, as the death rate was progressively high towards the end of the experiment.

125 eggs were hatched and the sex was determined for 115 (62 males and 53 females). 42 died during the experiment and

these were dissected as stated above and their sex determined. Of these 27 were males and 15 females.

For the first half of the experiment the females were more numerous than the males; the proportion of males then gradually increased until the final result gave 62 males and 53 females.

These results are of value only because the sex was determined for 92 per cent. of the entire generation, but the experiment must be repeated many times before the evidence can be of scientific value.

PHYSIOLOGICAL STUDIES ON PLANARIA.

III. OXYGEN CONSUMPTION IN RELATION TO AGE (SIZE) DIFFERENCES.

L. H. HYMAN,

Hull Zoölogical Laboratory, University of Chicago.

I. Review of Literature.

The present paper is a report of some determinations of the rate of oxygen consumption of small (young) as compared with large (old) planarians belonging to three different species. That the metabolic and other activities of small individuals are more rapid than those of large individuals of the same and different species is so well known from a number of independent investigations that further evidence appears almost superfluous. The majority of these investigations, however, deal with vertebrates, where a certain difficulty inherent in the nature of the material is encountered. This difficulty centers about the impossibility of finding a proper unit for comparison of animals of different sizes. Among the warm-blooded vertebrates, the metabolic rate has commonly been reckoned per unit of surface area on the necessity of taking into account loss of heat by radiation. But leaving out of consideration the difficulties of accurately measuring the surface, over which proceeding a controversy of long standing exists, the surface method of comparison is more or less meaningless in the case of cold-blooded animals. Nor is the method of comparison of metabolic rate per unit weight free from objection. A considerable part of the weight of the vertebrates and the higher invertebrates consists of skeletal material, connective tissue, fat, etc. The metabolic rate of such material must be relatively low, and further its proportion to the total weight must be different in animals of different sizes; nevertheless, these factors have not been considered in any of the researches on the subject, nor, as far as I am aware, has anyone attempted to make a correction for these inert portions of the body.

As Benedict and Talbot ('14) concluded after a study of the metabolism of infants, metabolism cannot be accurately measured either by weight or by surface, but only by the amount of active protoplasm. It is probably impossible to determine this but certainly in the soft-bodied lower invertebrates, weight is a more accurate index of the amount of active protoplasm than is the case in higher forms, where skeleton is present. For this reason, the metabolism of the lower forms deserves more attention than it has hitherto received from physiologists.

Among the early investigators of the effect of size differences on metabolic rate were Jolyet and Regnard ('77), who determined the rate of oxygen consumption per unit weight of a large number of fish, and some of the higher invertebrates. They found that among related groups small species consume more oxygen per unit weight per unit time than large species; and small individuals more than large individuals of the same species. Vernon ('95) confirmed this conclusion. He measured the rate of oxygen consumption per unit weight of large and small individuals of the same species, using as material a hydrozoan medusa, a scyphozoan medusa, two ctenophores, two gasteropods, and two pelagic tunicates. With a few exceptions the smaller individuals were found to consume relatively more oxygen than the large ones. Bounhiol ('02) working with twelve species of polychæte annelids, belonging to several different families, found that the carbon dioxide production was greater the smaller the individual. This was true for individuals of the same and of different species. On the other hand, Montuori ('13) was unable to come to any definite conclusion from his measurements of the oxygen consumption of a large number of species belonging to most of the aquatic groups. In some cases the small individuals respired relatively faster than large ones of the same species; in others the reverse was found; and in still others there was no relation between size and rate of respiration. The great irregularity of Montuori's results, as well as their disagreement with the work of others, suggests that he failed to control adequately the conditions under which the experiments were performed. Child

('19) found that small individuals of *Planaria dorotocephala* produce more carbon dioxide per unit weight than large ones, and this experiment is now regularly performed as a class experiment. Allen ('19) has shown that the rate of oxygen consumption of *Planaria agilis* per unit weight is greater the smaller the animals, and reports that Miss Wolf working in the same laboratory found the same to be true for the leech, crayfish, branchipus, may-fly nymph, and stone-fly nymph. It is also the case with dragon-fly nymphs, as shown by Mr. G. C. Hawk in this laboratory. According to Morgulis ('15), the oxygen consumed per gram per hour by flounders is in general greater for small than for large animals. Tashiro and Adams ('14) note that the carbon-dioxide production of the ganglionic cord of the heart of *Limulus* is relatively greater in small than in large cords. Nicolas ('18) found that the young leaves and stems of plants give off from $3\frac{1}{2}$ to 7 times as much carbon-dioxide per gram per hour as old leaves and stems from the same branch.

A considerable amount of labor has been devoted to this problem in the case of mammals, especially man. Rubner originally maintained that warm-blooded animals of different sizes produce the same amount of heat per unit surface, but subsequent investigations have shown that this point of view is erroneous. Thus Magnus-Levy and Falk ('99) clearly showed that the oxygen consumption and carbon-dioxide production is highest in children and decreases with age, as measured either per unit weight or per unit surface. If individuals of the same size and weight but of different ages are compared, the younger ones respire the faster. The authors conclude that the protoplasm of children has a definitely higher rate of respiration per unit weight than that of mature individuals. References to other work supporting these conclusions will be found in their paper. Recently Du Bois ('16) and Gephart and Du Bois ('16) have verified this earlier work. The graphs and tables presented by Du Bois show that the heat production as measured per kilogram of weight is highest in infants and decreases gradually; as measured per unit of surface, it is low in infants, increases rapidly during the first year, reaches a maximum between the ages of 1 and 6, falls rapidly to 20, and thereafter decreases more slowly. According to Benedict and Talbot ('14, '15), the metabolism of infants is about the same as that of adults, but the results were very variable. It seems highly probable that the metabolism of infants cannot properly be compared with that at other ages because: (1), the heat-regulating mechanisms of infants are known to be very imperfect; (2), infants commonly have relatively more fat than is present in normal individuals of other ages; and (3), the muscle tone of the voluntary muscles of infants must be lower than it is at other ages. Since a large part of the heat production of mammals originates in the voluntary muscles, this difference in muscle tone alone makes impossible any real comparison of the metabolism of infants with that of later stages of ontogeny when the muscles are in full use.

A few researches have been carried out on mammals other than man. Thus Slowtzoff ('03) working on dogs found that the oxygen consumption and carbon dioxide production per kilogram per minute is greater in small than in large individuals. A. V. and A. M. Hill ('13) determined the same relation in rats. Among birds, Bohr and Hasselbalch ('00) observed that the carbon dioxide production per kilogram per hour is considerably higher in newly hatched chicks than in the adult hen.

These researches are sufficiently numerous to establish the generalization that smaller (younger) individuals have a higher metabolic rate than larger (older) ones. The investigations on man clearly show that the difference is due to age and not to size. Age is also probably the determining factor in the metabolic difference found between large and small species, since, in general, species which grow to a large size must be older by the time they have attained that size than are adult individuals of small species.

How far back in the ontogeny can this generalization be carried? At what point in the ontogeny does the metabolic rate attain its highest value? Few researches have been carried out upon these points. The eggs of animals are probably cells of very low metabolic rate. After fertilization, the metabolic rate gradually rises, as development proceeds, up to a certain point and then falls. In the sea-urchin egg (*Arbacia punctulata*), the oxygen consumption was found to rise continuously up through late cleavage (determinations stopped at this point); and in the eggs of *Fundulus heteroclitus*, up to the time when the embryonic axis is established after which it fell, rising again later apparently as the result of functional activity (unpublished personal observations). Unfortunately in these cases, nothing is known of the metabolic rate of the adult. Hasselbalch ('00) and Bohr and Hasselbalch ('00) have measured the rate of carbon dioxide production and oxygen consumption per kilogram in chick embryos. Both were found to be very high in the earliest stages determined—five or six days,—much higher than those of the adult; the rates then fell rapidly. The carbon dioxide output fell to the ninth day, after which it was about contant, and about equal to that of the adult. The oxygen consumption fell to the eleventh day, then rose again, and on the sixteenth day was again considerably higher than that of the adult. Bohr ('00) compared the carbon-dioxide output of guinea-pig embryos with that of the mother. He did this by cutting open the uterus under anæsthesia, determining the carbon-dioxide output of the mother and fœtuses, then clamping the umbilical cords of the fœtuses, and again measuring the CO_2 output of the mother. The difference between the two measurements supposedly represents the carbon-dioxide production due to the embryos. Of the six experiments of this kind performed by Bohr, five showed the carbon-dioxide production of the fœtuses to be greater than that of the mother. The case where the youngest embryos (5.5 grs. each) were encountered gave a carbon-dioxide output very much higher than that of the mother; the next size (16.5 grs. each) gave less CO_2, but still at a rate considerably higher than that of the mother; while in the other four cases, with large fœtuses, the CO_2 production was slightly higher than that of the mother in three, lower in one.[1]

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[1] I wish to state that the conclusions which I have drawn after careful perusal of the papers of Bohr and Hasselbalch are somewhat different from those stated by the authors. They conclude, curiously enough, that the respiratory rate of the embryos of the hen and guinea pig is no higher than that of the adult, and anyone reading their conclusion alone would certainly be misled. The authors have either overlooked or ignored the fact, which their experimental data clearly show,

These researches indicate that the rate of respiratory exchange is very high at some certain stage in the embryonic development, this stage probably differing in different animals, and falls subsequently. Later it probably rises with increased functional activity. It certainly seems to me that the metabolic rate of embryos, especially vertebrate embryos, cannot vaildly be compared with that of post-embryonic stages, owing to the enormous differences in functional activity which exist between two such stages. The comparison of the metabolic rate of a chick embryo with an adult hen seems to me a simple absurdity when one reflects upon the difference in muscle tension alone at the two stages. The fact that the total metabolic rate of chick embryos was at no stage found to be less than that of the hen, certainly indicates that if cells of the same degree of functional activity could be compared in embryo and adult, the metabolic rate of the embryonic cells would be vastly the greater. The same criticism applies to cases where the young remain more or less helpless after birth or hatching.

In addition to these researches in which direct measurement of the rate of oxygen consumption or carbon dioxide output of young and old animals have been made, a considerable mass of data is available in which another method was employed. This is the direct susceptibility method extensively used in this laboratory by Child and others; it consists in observing the time of death of organisms in lethal solutions of various substances. We have brought forward a large amount of evidence[1] to indicate that the time of death in such solutions is an index of metabolic rate, individuals of higher metabolic rate dying first. When individuals of different ages are compared by this method, it is invariably found that the time of death is shorter the younger the individual, *always providing that the same degree of functional activity is present in the animals which are being*

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that the rate of respiratory exchange in the youngest embryos with which they dealt very greatly exceeds that of the adult. It is only at certain later stages that the rates of the two are approximately equal. The authors seem to have an idea that the rate of respiratory exchange ought to be the same throughout development, whereas their own and other data show that it is high in early stages and declines as development proceeds. This decline is probably of the same nature as that which organisms undergo from birth to maturity.

[1] A general résumé of this evidence will be found in Child, '13.

compared. This age difference in susceptibility to toxic solutions has been observed in *Paramecium*, three species of *Hydra*, a number of colonial hydroids and hydromedusæ, several species of planarians, and several small aquatic oligochætes; and the rise in metabolic rate which is a feature of early development has also been demonstrated by this method.

Not only does the rate of respiratory exchange in organisms vary inversely with age but many physiological activities exhibit the same relation. Bert ('70) was among the first to observe this fact. He noted that the rate of respiratory movements is faster in small than in large individuals of the same and related species. Ducceschi ('03) studied the rate of movement of the fins, tail, fin membrane, and operculum of a large number of fish of different sizes; the rate of movement of the maxillipeds, abdominal appendages, and claws of several crustacea; and of the fins and mantle of some cephalopods; and found that it varies inversely as the size of the animal. Mayer ('06) and Child ('18) observed that the rate of contraction of the bells of medusæ is faster the smaller the animal. The number of respiratory movements per minute of *Octopus* is greater the smaller the individual (Polimanti, '13). In four species of sea-cucumber, Crozier ('16) observed that the pulsation of the cloaca is more rapid the smaller the animal. Hecht ('16, '18) noted that small Ascidia pass relatively more water through their siphons than large ones; and further that the rate of the heart beat in Ascidia varies inversely as the size of the animal. In man, of course, it is well known that the rate of the heart beat is fastest in the fœtus and decreases progressively with age. The rate of growth follows a similar law.

The results of the present experiments are in full accord with those of previous investigators. Small (young) planarians whether asexually or sexually produced, consume more oxygen per unit weight per unit time than large (old) ones.

II. Experiments with *Planaria dorotocephala*.

Planaria dorotocephala lives in spring-fed marshes in morainic regions near Chicago. It has never been found sexually mature in nature (although sexual maturity has been experimentally induced by Dr. Child in this laboratory), but maintains itself by means of asexual fission. In fission, the posterior portion of the body pulls away from the anterior end, regenerates a head and becomes a complete small worm. Such small worms correspond in all tests which have been made upon them, to young worms in species reproducing sexually.

Six experiments were performed in which the rate of oxygen consumption of such small worms, under 10 mm. in length, was compared with that of large worms, over 20 mm. in length. In all cases, both large and small individuals were taken from the same stock and had been kept under the same conditions previous to the experiment. No worms showing signs of recent fission or regeneration were used. The heads were cut off the day before the test was made in order to eliminate movement. Two successive determinations of the oxygen consumption of each lot of worms were usually made, the worms were then weighed, and the oxygen consumption per two hours per 0.5 grams weight then calculated.

The method of determining the rate of oxygen consumption and the method of weighing have been described in previous papers (Hyman, '19*a*, '19*b*).

The results of the six experiments are given in Table I. In all cases the smaller worms consume more oxygen per unit weight per unit time than the larger ones. The per cent. difference ranges from 18 to 55, and closer inspection of the table reveals that the amount of difference is correlated with the length of time which has elapsed since the last feeding. In experiment 4, where the small worms respire but 18 per cent. more than the large ones, only two days had elapsed since feeding, while in the other experiments, where three or four days had passed since feeding, the difference is greater. As shown in a preceding paper (Hyman, '19*b*), feeding greatly increases the rate of oxygen consumption by stimulating the digestive tract. Since the digestive tract is more extensive in large than in small individuals, the difference between such individuals is decreased by feeding. As I did not perform any experiments with worms deprived of food for longer than four days, I do not know whether further starvation would increase the difference between small and large

TABLE I.

COMPARISON OF THE RATE OF OXYGEN CONSUMPTION OF LARGE (OVER 20 MM.) AND SMALL (UNDER 10 MM.) INDIVIDUALS OF *Planaria dorotocephala*.

Size of Worm.	C.c. Oxygen Consumed in 2 Hours.	Weight in Grams.	Oxygen Consumed by 0.5 Grs. in 2 Hours.	Per Cent. Difference.
Exp. 1. Worms Collected March 12, Last Fed April 2, Tested April 5. Temp. 22.5° C.				
Large	0.18 0.20	0.316	0.30	
Small	0.24 0.29	0.325	0.40	33
Exp. 2. Worms Collected March 12, Last Fed March 14, Tested March 17. Temp. 22° C.				
Large	0.12 0.11	0.187	0.30	
Small	0.23 0.17	0.255	0.39	30
Exp. 3. Worms Collected Early Winter, Last Fed April 23, Tested April 26. Temp. 21° C.				
Large	0.09 0.08	0.179	0.23	
Small	0.14 0.13	0.216	0.31	48
Exp. 4. Worms Collected March 12, Last Fed April 30, Tested May 2. Temp. 21° C.				
Large	0.16 0.17	0.160	0.51	
Small	0.22 0.24	0.192	0.59	18
Exp. 5. Worms from Mixed Stock, Last Fed May 6, Tested May 10. Temp. 21° C.				
Large	0.13 0.12	0.146	0.42	
Small	0.17 0.15	0.123	0.65	55
Exp. 6. Worms Collected May 28, Last Fed June 6, Tested June 10. Temp. 22° C.				
Large	0.33	0.370	0.22	
Small	0.33	0.272	0.31	41

worms, but it is scarcely likely since the effect of feeding has almost completely disappeared in four days. In Allen's experiments (Allen, '19) with *Planaria agilis*, worms starved 27 days were used; and in that case the difference between the largest and smallest worms was about 40 per cent. (Table I., decapitated worms).

III. Experiments with *Planaria velata*.

Planaria velata lives in the Chicago region in temporary ponds, usually those that are passing into prairie, known to ecologists as "prairie ponds." Like the preceding species it is never found sexually mature, and reproduces exclusively by a peculiar asexual method. After the worms have attained a certain size, pieces drop off from the posterior end, surround themselves with mucus, and pass into an encysted condition. This process continues until the entire worm has formed a series of cysts. Within the cysts, the pieces undergo regeneration into complete worms of very small size, and these emerge from the cysts in about four weeks. This cycle is repeated as long as the ponds contain water; after the water dries up, the cysts remain quiescent until the following spring.

Table II.

Comparison of the Rate of Oxygen Consumption of Large and Small Individuals of *Planaria velata*.

	C.c. Oxygen Consumed in 2 Hours.	Weight in Grams.	Oxygen Consumed by 0.5 Grs. in 2 Hours.
Three Lots of Worms 10–12 mm. Long, before Encystment; Collected March 7, Last Fed March 10, Tested 13. Temp. 22° C.			
Lot *C*	0.42 0.52	0.745	0.31
Lot *D*	0.58 0.52	0.774	0.35
Lot *E*	0.41	0.593	0.34
Three Lots of Worms less than 4 mm. Long, Emerged during May from Cysts Formed by Above Lots of Worms; Fed Several Times after Emergence; Last Feeding June 6, Tested June 9. Temp. 22° C.			
Lot *C*	0.16 0.16	0.125	0.64
Lot *D*	0.16 0.16	0.127	0.63
Lot *E*	0.10 0.09	0.070	0.67

Owing to the nature of the life cycle of this animal, it was not possible to test the large and small worms simultaneously but the large worms were tested before encystment, the small ones after emerging from the cysts. Three lots of each size were used. The worms were all taken from the same stock. The results are given in Table II. The heads were not removed in these cases, since the worms were also used for another experiment.

The table shows that the small worms consume 100 per cent. more oxygen than the large worms. The greater difference in this case than with the preceding species is probably due to the greater reorganization involved in the production of young worms with this species. As shown in another paper (Hyman, '19*c*), the process of regeneration of itself brings about a great increase in the rate of oxygen consumption.

IV. Experiments with *Planaria maculata*.

The so-called species *Planaria maculata* lives in the eastern United States under stones in ponds and in the Chicago region on submersed vegetation. It is highly probable that these two are not the same species as *Planaria maculata* from the Chicago region has never been found sexually mature, while that from Massachusetts is sexually mature and lays capsules all summer long; further the behavior of the two in regeneration is quite distinct. The forms used in this experiment were collected from a pond at Falmouth, Mass., and the experiments were performed at the Marine Biological Laboratory, Woods Hole, Mass., I am indebted to the director, Professor F. R. Lillie, for a research room in this laboratory.

Sexually mature worms, young worms, and egg capsules were collected at Falmouth and brought to Woods Hole. The rate of oxygen consumption of the mature worms was tested with one exception soon after they were collected. The young worms collected, together with those which subsequently emerged from the capsules, were kept for some time and fed at short intervals on liver (mostly fish liver) until a considerable number of them were at hand, whereupon their rate of oxygen consumption was determined. In all cases the heads were removed at least several hours before the test.

The results are recorded in Table III. The young worms consume about 50 per cent. more oxygen than the sexually mature individuals.

TABLE III.

COMPARISON OF THE RATE OF OXYGEN CONSUMPTION OF SEXUALLY MATURE INDIVIDUALS (15 MM. OR LONGER) WITH THE SEXUALLY PRODUCED YOUNG (5 MM. OR LESS) OF *Planaria maculata*.

No. of Lot.	C.c. Oxygen Consumed in Test.	Weight in Grams.	Oxygen Consumed by 0.5 Grams in 2 Hours.
Four Lots of Sexually Mature Individuals, Collected July 10; First Three Lots Tested July 11; Fourth Lot Kept Until August 11, with Frequent Feedings, Last Feeding August 8, Tested August 11. Temp. 20° C.			
Lot 1	0.30 in 2 0.31 hours	0.700	0.21
Lot 2	0.32 0.32	0.677	0.23
Lot 3	0.30 0.26	0.680	0.20
Lot 4	0.09 0.07	0.152	0.17
Three Lots of Sexually Produced Young; Young and Capsules Collected July 10 Fed at Frequent Intervals; Last Feeding of Lots 1 and 2, July 28, Tested July 31; Lot 3, Last Feeding August 8, Tested August 11. Temp. 20° C.			
Lot 1	0.14 in 3 0.12 hours	0.101	0.32
Lot 2	0.11 0.11	0.091	0.32
Lot 3	0.16 0.18	0.117	0.28

V. CONCLUSIONS.

These experiments show that small or young planarians consume oxygen at a faster rate per unit weight than larger or older ones. As already stated, the carbon dioxide production is also inversely proportional to the size of the worms. It is true that experiments of this kind do not and cannot prove that the protoplasm of young animals actually has an intrinsically higher metabolic rate than that of older ones, for the reason that it is impossible to discover what part of the weight of an animal is active protoplasm and what part inert material. Nevertheless there cannot be any reasonable doubt that the metabolic rate is inversely proportional to age. It would be difficult to suggest any other explanation for many of the facts cited in this paper. namely, for the faster rate of respiration, faster heart beat, and more rapid rate of other physiological activities of young as compared with older organisms. The fact further that the susceptibility of young animals to a number of toxic substances is greater than that of old could scarcely be supposed to be due to a greater percentage of inert materials in the older individuals,

An interesting point brought out in these experiments is that the difference between the asexual and the sexual young and their respective adults is of about the same magnitude, when considered the same length of time after feeding. Worms produced by fission are therefore as truly "young" as those which develop from the egg.

In previous papers of this series (Hyman, '19*b*, '19*c*), it was shown that planarians which have been starved seven or eight weeks and pieces of planaria which have undergone regeneration have a much higher rate of oxygen consumption than ordinary fed worms, all tests being made, of course, a few days after feeding. Starved, regenerated and young worms therefore have this physiological characteristic in common: their metabolic rate is higher than that of large fed worms. That of starved ones is highest, regenerated ones next, and young, when produced from the egg or simple fission, least. It therefore appears that the metabolic rate of reduced forms depends primarily upon the amount of reorganization involved in their production, and is proportional to the degree of reorganization which has taken place. As a further illustration of this may be cited the much higher metabolic rate of the asexual young of *Planaria velata* than those of *P. dorotocephala*, presumably because much more extensive changes are involved in giving rise to the former. The evidence presented in these papers clearly supports the view which has been long maintained by Child—that such reorganizations due to whatever cause are rejuvenating transformations, restoring the organism to a physiological condition resembling that of the young.

V. SUMMARY.

1. The young of *Planaria dorotocephala* produced by simple fission were found to consume 15 to 55 per cent. more oxygen than large worms, the difference depending upon the length of time which had elapsed between the last feeding and the time of testing.

2. The young of *Planaria velata*, produced by an asexual process involving regeneration and a high degree of reorganization of the body, consume about 100 per cent. more oxygen than the worms from which they come. The greater difference in this species is undoubtedly associated with the method by which the young are produced.

3. The sexually produced young of *Planaria maculata* consume about 50 per cent. more oxygen than the sexually mature worms. There is thus no significant difference between sexual and asexual young, when the latter arise by ordinary simple fission.

4. This result, that young worms have a higher metabolic rate than old ones, is in accord with a considerable body of literature on other forms leading to the same conclusion; and confirms the work previously done in this laboratory upon this same point by other methods.

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ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION.

III. ACTION OF POTASSIUM CYANIDE ON SOME CŒLENTERATES AND ANNELIDS.

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In this paper are presented some further data concerning the effect of potassium cyanide on the rate of oxygen consumption of animals. In view of the importance of this substance as a reagent in physiological experiments, and since tests of this kind have been made upon comparatively few animals, it has seemed worth while to obtain data upon some representatives of groups in which the action of cyanide has not yet been determined. No experiments have, to my knowledge, been performed upon annelids, and only one cœlenterate has been tested, namely *Gonionemus*, in which form Loeb and Wasteneys ('13) showed that potassium cyanide decreases the oxygen consumption to a considerably greater extent than does ethyl urethane, although both produce the same degree of anæsthesia.

The literature dealing with the chemical, physiological and pharmacological properties of the cyanides and related substances has been extensively reviewed in the second paper of this series (Hyman, '19), and will therefore not be restated here. Briefly, it may be said that this group of substances has been shown to depress many physiological processes; and to decrease the rate of oxygen consumption or carbon dioxide output or both of the following living materials: yeast, a mould *Aspergillus*, a sponge *Suberites*, *Gonionemus*, *Planaria*, a beetle *Passalus cornutus*, embryos of *Fundulus*, several mammals, sea-urchin eggs, red blood corpuscles of geese, isolated mammalian kidneys, the frog's heart, minced beef liver, minced horse, beef, and pigeon muscle, and minced horse brain. In most of these cases it was shown that the effect was reversible. Since that review was written, two additional papers have come to my attention, those of Allen ('19) and of Evans ('19). Allen's results and conclusions regarding the effect of potassium cyanide on the rate of oxygen consumption of *Planaria* are about the same as my own, except for one or two points; thus Allen states that the oxygen consumption of *Planaria* cannot be reduced to less than 20 per cent. of the normal by cyanide, while I found in many cases, reduction to 10 to 15 per cent. of the normal. Evans's interesting paper contains some further references to the literature, confirms some previous work, and presents a number of new facts. Thus Evans finds that cyanide decreases markedly and reversibly the oxygen consumption of the cat, confirms the older statements that oxygen is just as readily dissociable from the blood in cyanide poisoning as from normal blood; and shows a striking similarity between the behavior of smooth, skeletal, and heart muscle, and nerve and nervous centers in the presence of cyanide and in lack of oxygen. Evans concludes that the cyanides "appear to exert all of their physiological effects by reason of the anoxæmia which they produce," and favors the view that they act by uniting chemically with some tissue constituent.[1]

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[1] Comment would seem to be required on Evans's remark (p. 23) that the observations of Lund and Herwerden seem to be incompatible with Child's conception of metabolic gradients. Lund's results have been considered elsewhere (Child, '19). Herwerden ('18) says that the buds and young of *Hydra* are not more susceptible to cyanide than the parents. Perusal of her paper shows, however, that she has compared only three or four pairs of individuals and has observed the *disintegration of the tentacles only*, having removed the cyanide when this occurred. Now, we have clearly shown (Child and Hyman, '19) that the buds of *Hydra* are not comparable with the parents *until they have the same degree of muscular activity*, and further that the susceptibility of the tentacles is also *dependent upon their degree of activity*. Miss Herwerden is mistaken in her conclusion. We have compared dozens of young and adult *Hydras*, belonging to three species, and have always found that the young are more susceptible to cyanide *when comparable degrees of muscular activity* exist. It is true that the difference is *least* in the case of the tentacles but even there the tentacles of the young are slightly more susceptible. Neither are Miss Herwerden's statements about the susceptibility of the Daphnid heart entirely correct. I have found that the smallest individuals in a *Daphnia* culture are the most susceptible of all (both as to heart and entire animal); that the susceptibility decreases with increasing size up through the size when the females are producing their first parthenogenetic broods; but from that time on, the relation is reversed, so that the largest and oldest individuals in the culture, which have produced many parthenogenetic broods, are more susceptible than

In the experiments to be reported in this paper it was not my purpose to make a complete study of the action of cyanide on oxygen consumption in these animals, since I had already done that in the case of a sponge and *Planaria*, but merely to show that cyanide in non-lethal doses reversibly decreases oxygen intake. The experiments on the marine forms were performed at the Marine Biological Laboratory, Woods Hole, Mass.; those on the fresh-water forms at the University of Chicago. I am greatly indebted to Professor F. R. Lillie for working space at the first-named institution.

The methods employed were identical with those used in previous experiments on this subject and will be found described in full in a former paper (Hyman, '19). Briefly, the animals to be tested were placed in Erlenmeyer flasks or wide-mouthed bottles of 500 to 600 c.c. capacity, these were filled air-tight with water, and the animals allowed to respire in them for a convenient length of time. A sample was then withdrawn, and this and a sample of the original water used were analyzed for oxygen content by Winkler's method, the difference between the two samples representing the oxygen consumed by the animals. Two separate determinations of the oxygen consumption in normal water were made, potassium cyanide was then added to the water and two more determinations in the presence of cyanide carried out. The animals were then washed in several changes of water, and their oxygen consumption in normal water tested again a day or two later. Throughout each experiment, the receptacles containing the animals were immersed in a water-bath, the temperature of which was kept constant to 0.5 degrees.

The possibility of iodine absorption by substances emanating from the organisms was again considered, and was tested in the case of two or three of the animals employed. Standard iodine solution was added to water which had been standing on the animals for at least one hour, and the iodine then titrated back

half-grown individuals. It would thus appear that in these animals parthenogenetic reproduction is accompanied by some degree of rejuvenescence. Green's recent work on *Simocephalus* (Biol. Bull., Aug., 1919) supports this suggestion, since he found that these animals reproduce sexually early in ontogeny, and subsequently the same individuals begin parthenogenetic reproduction, a reversal of the order usually observed in parthenogenetic forms.

with thiosulphate. No loss of iodine was found, and indeed, this possible source of error in the Winkler method would seem to have been greatly overrated (*cf.* Hale and Melia, '13).

EXPERIMENTS ON PENNARIA.

Pennaria tiarella, a common colonial hydroid of the Atlantic coast, was selected as a representative of this group of cœlenterates. Large quantities of freshly collected material were available. Clean branches, free from visible plant growth, were cut off, *Caprella* and other small animals which commonly live on such colonies carefully removed, and the branches were then placed in wide-mouthed bottles, and treated as described above. The animals were always used within a few hours after they were collected.

TABLE I.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Pennaria tiarella*.

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 18, 19° C.			Aug. 23, 22° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.55	0.56	0.43	0.94	0.78	1.21
Second hour normal	0.62	0.56	0.43	0.95	0.74	1.20
	1/25000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.38	0.38	0.27	0.52	0.45	0.60
Second hour KNC	0.32	0.35	0.25	0.36	0.34	0.54
Per cent. decrease	41	35	39	54	49	53

Six different lots of *Pennaria* were tested in this manner, as to their normal rate of oxygen consumption and the rate in 1/10000 and 1/25000 mol. potassium cyanide. The results are given in Table I. In all cases, the rate of oxygen consumption is reduced in the presence of cyanide. Unfortunately in the case of this animal it could not be determined whether the effect was reversible, since *Pennaria* deteriorates rapidly in the laboratory; within twenty-four hours, the majority of the hydranths have either fallen off or have lost their normal appearance.

Since *Pennaria* exhibits very few movements, it is not possible that differences in muscular activity could account for the decreased oxygen intake in the presence of cyanide. As far as could be determined, the hydranths were as fully expanded in the presence of cyanide as in normal sea-water.

Experiments on Metridium.

Metridium marginatum was selected as a representative of the Anthozoa. The individuals used had probably been kept in float cars for some time. Medium-sized individuals were placed in wide-mouthed bottles of about 600 c.c. capacity, one in each, and left there undisturbed until they had attached themselves and expanded fully. It was found that the animals soon became accustomed to such an environment and could be handled and would submit to change of water without contracting. Since the oxygen consumption of sea-anemones in all probability varies with the degree of expansion, note was always made of the degree of expansion during the experiments. In experiments 1 and 3, the animals were slightly contracted during the exposure to cyanide but in the other six experiments they were fully expanded throughout. Differences in state of muscular activity are therefore not responsible for the observed results.

The eight experiments which were performed upon *Metridium* are reported in Table II. A marked and reversible decrease in oxygen consumption in the presence of cyanide was found in all cases.

Table II.

Action of Potassium Cyanide on the Oxygen Consumption of *Metridium marginatum*.

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.
Temp. and Date	Aug. 12, 21° C.			Aug. 10, 21.5° C.		Aug. 13, 20° C.		
Oxygen Consumed, Cubic Centimeters per Hour.								
First hour normal	1.33	1.19	1.13	1.66		1.06	1.01	0.97
Second hour normal	1.09	1.05	1.03	1.94	1.67	1.12	1.19	1.31
	1/5000 Mol. KNC.			1/10000 Mol.		1/25000 Mol.		
First hour KNC	0.48	0.56	0.56	1.24	1.25	0.81	0.70	0.66
Second hour KNC	0.49	0.59	0.42	0.89	0.70	0.70	0.76	0.59
Per cent. decrease	60	49	55	41	58	32	34	46
Recovery	1.09	1.10	1.14	1.68		1.08	1.18	1.20

Experiments on Polychætes.

Experiments were performed on three common polychæte annelids of the Atlantic coast—*Nereis virens*, *Arenicola cristata*, and *Chætopterus pergamentaceus*. Of the three species, *Chætopterus* was found to be the most favorable for this kind of experimentation, as it is not a very active animal. No difference in amount of activity in normal and cyanide-containing sea-water was observed. *Arenicola* was fairly favorable for the purpose as it also is relatively inactive, and here again there was no significant difference in activity throughout the experiments; indeed, it seemed to me that the respiratory movements were more pronounced in the presence of cyanide than in normal sea-water. On the other hand, the results with *Nereis* were not very satisfactory owing to the restlessness of the animals. In experiments 1–3, Table V, the animals were considerably more active in normal sea-water than they were after cyanide had been added, and hence the decrease observed in cyanide was due in part to diminished motor activity. In experiments 4–6, Table V., however, differences in motor activity were not present and have not contributed to the result.

A further difficulty was encountered in the case of *Nereis*. It was observed that both the degree of activity and the rate of oxygen consumption of *Nereis* diminished when it was kept in the laboratory. Experiments 1–3 were performed on freshly collected individuals and it will be noted that the oxygen consumption of these individuals is much higher than in the other cases, and further that the recovery figures, obtained upon them twenty-four hours later, are considerably lower than the original figures. This is not due to the exposure to cyanide since individuals from the same collection which had not been subjected to cyanide showed the same decrease in oxygen intake after a day in the laboratory. It is probable that this decrease is due in large part to starvation. I have observed it in the case of a number of other animals also, and my experience indicates that animals which have been kept for a few days after removal from their natural environment are preferable to freshly collected material for experiments of this kind. This difficulty was not encountered in the case of *Chætopterus* and *Arenicola* since it happened that the animals had been on hand in the collecting department for some time before I obtained them.

Since the first experiments showed that some little time was required for the penetration of even relatively concentrated solutions of cyanide into these animals, presumably because of their thick body walls, the practice of leaving them for an hour or two in the cyanide solution before carrying out the test was adopted.

TABLE III.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Chætopterus pergamentaceus.*

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.	6.
Temp. and Date	Aug 3, 20.5° C.			Aug. 3, 21° C.			Aug. 5, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.									
First hour normal	0.48	0.40	0.56	0.44	0.36	0.45	0.47	0.35	0.44
Second hour normal	0.33	0.40	0.48	0.40	0.36	0.44	0.49	0.32	0.43
	1/2000 Mol. KNC (Two Hrs. Before Test).			1/5000 Mol. KNC (Two Hrs. Before).			1/10000 Mol. KNC (One Hr. Before).		
First hour KNC	0.20	0.13	0.23	0.23	0.16	0.27	0.41	0.24	0.34
Second hour KNC	0.14	0.16	0.21	0.14	0.16	0.22	0.41	0.22	0.33
Per cent. decrease	59	64	58	56	56	45	15	32	23
Recovery	0.34	0.48	0.50	0.48	0.34	0.43			

TABLE IV.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Arenicola cristata.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 2, 20.5° C.			Aug. 1, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.42	0.39	0.67	0.42	0.48	0.43
Second hour normal	0.34	0.38	0.63	0.44	0.47	0.56
	1/1000 Mol. KNC (Two Hrs. Before).			1/2000 Mol. KNG (Two Hrs. Before).		
First hour KNC	0.27	0.30	0.46		0.22	0.31
Second hour KNC	0.20	0.26	0.46	0.27		0.30
Per cent. decrease	39	28	39	35	54	39
Recovery	0.49	0.31	0.71	0.42	0.48	0.43

The results with *Chætopterus* are presented in Table III., with *Arenicola* in Table IV, and with *Nereis* in Table V. One or two individuals, depending on size, were placed in each flask. For reasons already given, experiments 1–3, Table V., on *Nereis* were not very satisfactory but the other experiments on these polychætes leave no doubt that cyanide brings about a reversible decrease in oxygen consumption.

TABLE V.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Nereis virens.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 5, 22° C.			Aug. 6, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.80	0.65	0.65	0.30	0.36	0.29
Second hour normal	0.78	0.55	0.64	0.23	0.23	
	1/5000 Mol. KNC (Two Hrs. Before Test).			1/1000 Mol. KNC (One-half Hour Before).		
First hour KNC	0.14	0.11	0.11	0.05	0.05	0.08
Per cent decrease	83	82	83	83	84	73
Recovery	0.32[1]		0.48[1]	0.34		0.29

EXPERIMENTS ON LEECHES.

The leeches used in these experiments were *Hæmopis marmoratis*, and *Herpobdella punctata*, chiefly the latter. They were obtained from ditches near Wolf Lake, Indiana, and subjected to experiment shortly after they were brought into the laboratory. Two or three individuals were placed in each flask. As in the case of the polychætes, it was impossible to eliminate movement, but the degree of activity was about the same in both normal and cyanide-containing water. The results are given in Table VI. Experiments 4 and 7 were performed upon *Hæmopis*, the others on *Herpobdella*.

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[1] Failure to recover original rate of oxygen consumption not due to cyanide, see text.

TABLE VI.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF LEECHES (*Hæmopis marmoratis*, EXPS. 4 AND 7, AND *Herpobdella punctata*, REMAINING EXPS.).

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Temp. and Date	April 3, 21° C.			Mar. 22, 21° C.			April 1, 21° C.			
Oxygen Consumed, Cubic Centimeters per Hour.										
First hour normal	0.15	0.22	0.19	0.51	0.31	0.28	0.51	0.21	0.24	0.20
Second hour normal	0.16	0.24		0.51	0.30	0.25	0.48			
	1/5000 Mol. KNC.			1/10000 Mol. KNC.			1/25000 Mol. KNC.			
First hour KNC	0.01	0.07	0.04	0.17	0.17	0.15	0.29	0.08	0.17	0.14
Second hour KNC	0.04	0.07	0.06	0.13	0.14	0.11	0.26	0.09	0.14	0.13
Per cent. decrease	84	70	74	73	51	51	45	60	36	33
Recovery	0.17	0.28	0.23		0.26	0.33		0.16	0.23	0.19

EXPERIMENTS ON OLIGOCHÆTES.

The forms used in these experiments were an aquatic earthworm, *Helodrilus tetædra*, one of the megadrilous oligochætes, and *Lumbriculus inconstans*, one of the microdrilous oligochætes. Both of these annelids live in temporary pools in the woods in the dune region of Indiana; the former occurs in the larger pools and also in permanent ponds, while the latter species has never been found in permanent bodies of water. They were collected near Clarke, Indiana. Both species are highly thigmotactic, being found in their natural habitat entwined among the branches of the moss which commonly grows in these pools or lying between layers of dead leaves, their posterior ends usually protruded for respiratory purposes. As the animals are very restless when their bodies are not in contact with objects, an attempt was made to quiet them by placing a small amount of thoroughly washed cotton in the experimental flasks with the worms for some hours preceding the tests. This procedure was entirely successful with *Helodrilus;* the worms entwined themselves among the cotton fibers and remained perfectly quiet throughout the experiments. It was possible to quiet the majority of the individuals of *Lumbriculus* in this way also, but a few individuals would always continue to crawl about. No difference, however, was observed in the degree of activity in normal and in cyanide-containing water.

The results with *Helodrilus* are presented in Table VII. and with *Lumbriculus* in Table VIII. As these worms are rather small, a large number of individuals was used in each experiment. The results are the same as in the case of the other animals tested, a reversible decrease in oxygen intake when cyanide is present.

TABLE VII.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Helodrilus tetædra.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	June 3, 23° C.			June 2, 23° C.		
Oxygen Consumed, Cubic Centimeters in Two Hours.						
First hour normal	0.13	0.16	0.16	0.17	0.18	0.19
Second hour normal	0.13	0.19	0.15	0.16	0.22	0.22
	1/5000 Mol. KNC.			1/10000 Mol. KNC,		
First hour KNC	0.05	0.08	0.09	0.13	0.16	0.11
Second hour KNC	0.06	0.06	0.07	0.09	0.12	0.08
Per cent. decrease	59	60	49	30	30	54
Recovery	0.14	0.16		0.14	0.19	0.16

TABLE VIII.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Lumbriculus inconstans.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Oct. 17, 21° C.			Oct. 15, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.44	0.47	0.37	0.58	0.58	0.46
Second hour normal	0.45	0.41	0.29(?)	0.57	0.61	0.49
	1/5000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.20	0.24	0.18	0.30	0.28	0.21
Second hour KNC	0.14	0.16	0.14	0.28	0.26	0.19
Per cent. decrease	62	55	52	50	55	56
Recovery	0.38	0.42	0.42			

Summary and Conclusions.

1. The normal oxygen consumption and the oxygen consumption in the presence of various concentrations of potassium cyanide was tested in the case of *Pennaria*, *Metridium*, *Nereis*, *Chætopterus*, *Arenicola*, two species of leeches, and two species of aquatic oligochætes. In all cases, numbering about seventy experiments (not all of which are reported), the oxygen consumption was markedly decreased in the presence of cyanide.

2. This decrease was not due to differences in muscular activity, since in some of the experiments it was possible to keep the animals entirely quiet throughout, while in the others, with three exceptions noted in the text, the animals were equally active in both the normal and the cyanide-containing water. None of the concentrations of cyanide used caused any visible anæsthesia, within the short time periods during which the animals were exposed to them.

3. The decrease was reversible, the oxygen consumption returning to approximately the original value when the cyanide was washed out of the animals. In no case were the animals injured in any way. Most of them were kept for a considerable length of time after the experiments were completed and were entirely normal in behavior and appearance.

4. As was found to be the case in previous experiments with cyanide, the percentage of decrease is absolutely greater, the more concentrated the cyanide solution, but the more dilute solutions are relatively more effective.

5. These results are in accord with previous experiments on the action of cyanides and justify the use of the cyanides as depressing agents.

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